

nature*May 8, 1975*

For those in peril on the sea

H. RUSSELL BERNARD, an associate professor of anthropology at West Virginia University, has spent six weeks aboard American research vessels under the sponsorship of the Office of Naval Research (ONR) studying the relations between crew and scientists. At the end of it he declares that "when they are not working together, they are working at staying out of each other's way. Physical isolation of crew and scientists was achieved by an elaborate time and space-sharing scheme . . . [which] may be seen as a manifestation of a gulf in values and behaviour in general". I wish the ONR had asked me; for half the price of placing an anthropologist on a ship I would have been happy to tell them that in the average research ship there are large spaces, even whole decks, that are no-go areas for either scientists or seamen. There are no notices saying "keep out"; the atmosphere is just not conducive to visitation.

Now there is, without doubt, good cause for someone to worry about scientist-crew relationships on oceanographic expeditions, one of the most expensive ways of doing research. The memory of the throwing overboard of Antarctic specimens kept in a crew's beer freezer is too vivid for complacency, and the crew of a ship cannot (with a few distinguished, even famous exceptions) be regarded as laboratory assistants or technicians in the land-based sense of the word. But what is really striking on most research cruises is not how the scientists and crew come to some sort of truce, but how deeply the scientists can dislike each other.

The reasons are not difficult to find. It is a certain sort of person who chooses to spend month after month at sea, and yet when he gets there he finds himself in the closest proximity, sixteen hours a day, with a similar sort of person, whose soggy cornflakes at breakfast are likely, in high seas, to end up in his lap. Data collection goes on all night, so each will have regular opportunities to awake the other in an indelicate manner at a hideous hour. Many experiments can only be carried out to the exclusion of all others, leading to endless bickering over the allocation of time. The mixture of disciplines—meteorologists, chemists, geologists, biologists, fluid dynamicist—which (on land, at least) is supposed to be good for science leads to blank incomprehension and a

studied inability to see the point of anyone else's work. Some sea-goers work frantically all day and all night and cannot tolerate those who only appear for an hour a day. And one in ten of all oceanographers suffers from seasickness, frequently brought on by ships whose stability seems questionable. In the midst of all this stands the chief scientist, with problems not unlike those of an ice-hockey referee, and with the added complication that captains are always keen to get the ship back to port a day early.

The manifestations of these tensions are often bizarre. There is much practical joking of a pointed sort—a meteorologist may find his cabin completely filled with one of his balloons, and the presence of empty explosives cases allow for some pretty realistic and scary scenarios. The fighting is usually symbolic (water-filled balloons being very popular) but does on occasions end in a punch-up. The presence of so many tape recorders allows the alert discreetly to record the violent arguments that break out now and then, for replay at a quieter time. And accusations about the theft of tools never cease. Every oceanographer takes to sea with him a couple of dozen screwdrivers covered in complex identification marks and yet returns empty-handed and convinced that all his colleagues' locked toolboxes are stuffed with his property. When a research vessel goes to the breaker's yard, out of every crevice must come a screwdriver and a pair of pliers.

Amazingly, this physically unpleasant and emotionally conflict-laden environment not only exerts such a powerful attraction that sailor-scientists come back for more but also seems particularly fertile for the generation of new ideas. The best thinking in oceanographic science is often done on board ship in spare time rather than in the office back home, even though the best reception it is likely to get among one's shipmates is polite boredom. Perhaps a good way to revive some flagging scientific enterprises would be to transfer them lock, stock and barrel to a modest-sized converted trawler, to send them out to the mid-Atlantic and to require them to operate twenty-four hours a day.

There is plenty of sociological research left for the ONR to support. □



The little nipper who cost the South a fortune

Colin Norman reports on a threat to call a halt to a long and largely unsuccessful attempt to eradicate the fire ant.

THIS is an account of a fruitless attempt to block an invasion, of political manoeuvrings and fiscal blackmail, of chemical warfare and environmental destruction, and of open dispute between two factions in the federal government. Underlying the whole sorry affair are a variety of scientific issues, but they have generally taken a back seat.

The invasion began in 1918 when a small black ant, known as *Solenopsis richteri*, established a colony near Mobile, Alabama, after hitching a ride on a shipment of fruit from South America. Since then the ant and its red cousin *Solenopsis invicta* has invaded nine states from Texas to North Carolina, resisted a fearful onslaught of insecticides, soaked up \$115 million in public money and stirred up a massive controversy.

For the past 18 years, the Department of Agriculture, in concert with the infested states, has been attacking the ants with a variety of chemicals, with the result that the programme has the dubious distinction of being the most expensive single pest-control effort ever carried out in the United States. But last month, Earl Butz, the Secretary of Agriculture, announced that the federal government is finally cutting its losses and will cease bombarding the ants with pesticides on June 30.

The announcement is probably more of a threat than a promise, however, for Butz's statement seems designed to bring political pressure to bear on the Environmental Protection Agency (EPA) to lift restrictions on the pesticide used to attack the ant. "Continuing restrictions placed on the pesticide", Butz said, "have finally made the program completely unworkable", and for good measure he added that the ants "could be totally eradicated with only negligible effects on the environment" if only the EPA were not so diligent in enforcing its regulations.

Officials of the EPA were clearly caught by surprise, for in 1971 the Department of Agriculture decided that for various reasons, including cost, a programme to eradicate the ants was no longer feasible. Future efforts would therefore be aimed at preventing the pest from spreading, the Department

said. Nevertheless, Butz's statement has already promoted an outcry in the deep South, and some powerful southern Democrats are beginning to ask questions on Capitol Hill. The battle, clearly, is far from over.

Why has this insect provoked such a costly and seemingly futile campaign? The chief reason is that it has a nasty habit of attacking anybody who comes near it, inflicting a painful sting for which the creature has earned the name of 'fire ant'. It also builds mounds, sometimes reaching a height of about 3 feet, which have hard crusts and present a hazard to farm implements.

About 10,000 people a year were treated in Alabama, Georgia and Mississippi for fire-ant stings in 1969-71. Secondary infections and allergic reactions are the most common problems, although there have only been one or two reports of death from severe allergic reactions—in that respect, the ant is much less of a problem than wasps or bees. A committee which studied the fire-ant problem in 1972 reported that it is "of virtually no importance as a primary pest of crops, livestock or cattle" and in fact it may in some instances be helpful in killing off crop pests. The committee said, however, that the fire ant can harm agriculture by stinging farmworkers, to the extent that they will sometimes refuse to work in heavily infested fields.

The fire ant is clearly a great nuisance, but whether it merits a full scale chemical assault is, however, another matter. In any case, in view of the failure of past efforts to control the ant's spread, Butz's decision to terminate the programme seems like a prudent financial decision at a time when the Ford Administration is trying to reduce public expenditures.

The invasion began slowly at first, encompassing only about 2,000 acres by 1932. In the next 15 years, however, the fire ant had spread to about 2 million acres and by 1959 its territory had widened to 26 million acres. The federal government and the states began their effort to combat the ant in 1957, but the insect promptly responded by expanding its territory to 126 million acres between 1959 and 1969, which makes the anti-invasion strategy about as effective as the federal government's containment activities on the other side of the world. And, like the generals in Vietnam, the field commanders in the fire-ant programme were constantly promising that the end would soon be in sight.

In the early stages, heptachlor and dieldrin were used to bombard the fire

ant at a cost of some \$2 million a year to the federal government; a similar amount was supplied by the treasuries of the southern states. But, in 1962, the campaign switched to a new weapon—a chlorinated hydrocarbon called Mirex which was reckoned to create fewer environmental hazards and to be more effective than the other pesticides.

Described in one report as having "many of the requirements for an ideal control chemical for the ant", Mirex is applied in very small amounts—about 1.7 g per acre—dissolved in soyabean oil and spread on corn-cob grits. The grits are dropped on to infested fields by aircraft, and are taken up by foraging ants which carry the pesticide back into the mound, where the poison eventually wipes out the entire colony.

Although there had been a few attempts within the federal government to bring a halt to the fire-ant programme during the mid-1960s, the first major challenge came in 1970 in the form of a court suit brought by the Environmental Defense Fund (EDF), a Washington-based environmental organisation. Following reports that Mirex residues had turned up in a wide variety of organisms, particularly crustaceans and other aquatic wildlife, the EDF brought a suit to halt the programme. The immediate result was that on March 17, 1971 the EPA notified the manufacturer of Mirex—the Allied Chemical Corporation—of its intent to cancel registration of the pesticide. That first formal step to remove the chemical from the market was designed chiefly to open up a long investigation of Mirex, which was still going on when the Department of Agriculture made its announcement last month.

The first stage in the investigation was the appointment of a scientific advisory committee, which recommended to the EPA in 1972 that use of Mirex be continued to control the ants. The committee said, however, that the programme should not be aimed at eradication because "the environmental impact of such treatment is too poorly understood and the expense too great to justify a program of such magnitude". The EPA, therefore, decided to prevent the use of Mirex in coastal areas, heavily wooded areas and near inland waters. It also limited use of the pesticide to one application a year. Finally, the EPA began public hearings on the environmental impact of the programme in 1973, and those have been going on intermittently for the past fifteen months.

Although the EPA's regulations merely reflected what the Department of Agriculture was already doing in 1973, it was those restrictions which Butz condemned last month for crippling the fire-ant programme.

Why has the effort dragged on for so long, in spite of little success? The answer lies partly in the complicated politics of the deep South. For one thing, the \$68 million that the federal government has so far pumped in to the programme (which has been augmented by \$47 million from the states themselves) has provided a welcome addition to the coffers of state agriculture departments. And for another, the programme has powerful supporters in Washington, notably Jamie Whitten, chairman of the House appropriations subcommittee which handles the budget of the Department of Agriculture, and J. Phil Campbell, the Under Secretary of Agriculture. Whitten comes from Mississippi and Campbell comes from Georgia, both of which are heavily infested with fire ants.

Whitten's support for the programme in fact twice saved it from the axe in the mid-1960s, when the Bureau of the Budget tried to eliminate it. Whitten was furious and eventually increased the budget for the programme instead of eliminating it. By 1974, federal funding had passed the \$7-million-a-year mark, and the Administration had asked for \$9 million for the next year.

The EPA's surprise at Butz's decision to pull out of the fire-ant programme is evident from a letter sent to Butz by John Quarles, Deputy EPA Administrator, a few days after the announcement

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was made. Noting that the Department of Agriculture had "abandoned the concept of eradicating the fire ant prior to the imposition of EPA restrictions on the advice of scientists that this effort would be financially and logistically infeasible", Quarles said that the announcement "tends to bring undeserved criticism to [the EPA] when other considerations appear to have played a larger role" in the decision to terminate the programme.

Replying to the letter, Under Secretary Campbell said that "we have chosen to exercise our prerogatives responsibly by suspending the program until we can operate free of crippling restrictions. We ask that, as you exercise your responsibility, you take into consideration that to be environmentally sound, the program must

eliminate the fire ant as a pest".

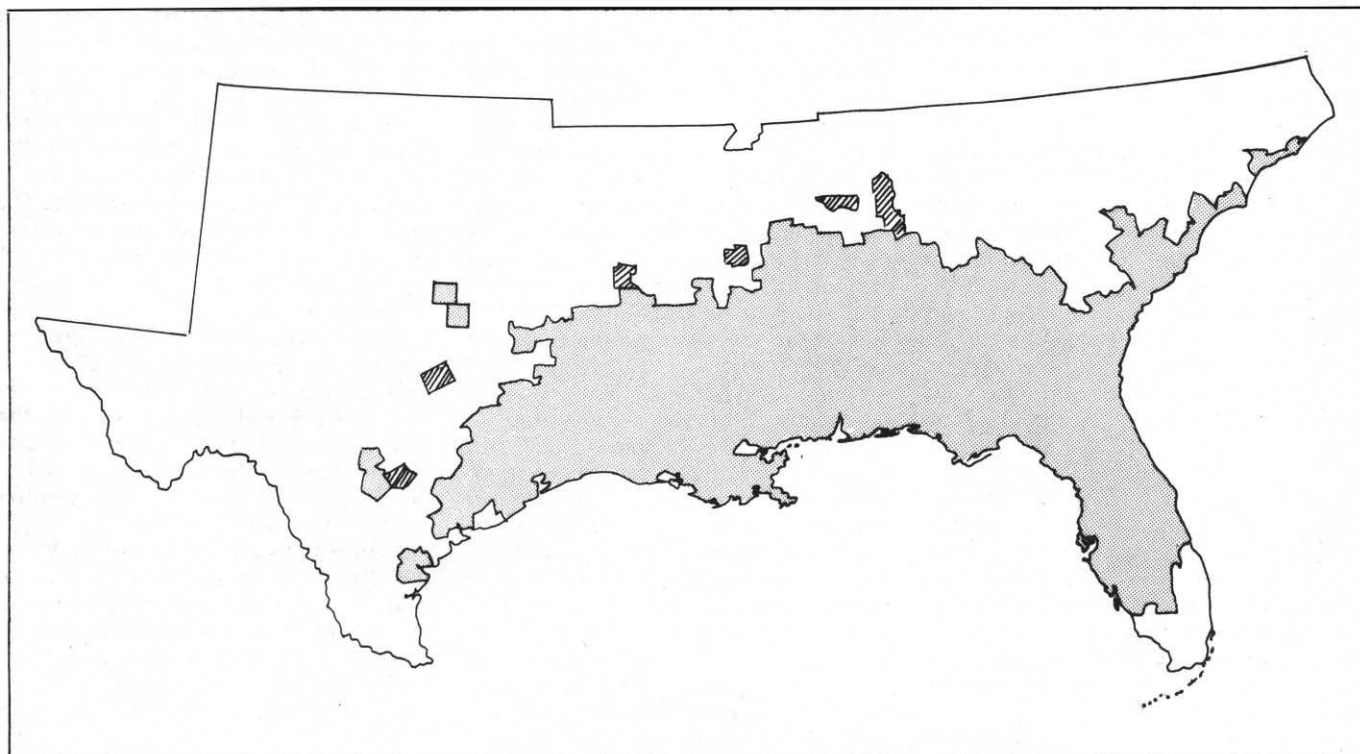
Asked last week what would be required to eliminate the fire ant entirely, scientists at the Department of Agriculture said it would probably take three applications of Mirex in an eighteen-month period over the entire infested area, including coastal regions. Since the federal government last year spent about \$1 per acre on the programme, complete eradication would run into hundreds of millions of dollars.

The department's belief that eradication is possible is based on three large-scale trials carried out in Georgia, Florida and Mississippi in 1967, where almost total eradication of the ant was achieved after three applications of Mirex. Opponents of the programme point out, however, that the areas rapidly became reinfested, and that if such a programme is carried out on a larger area, the potential destruction of non-target species would be very large.

Nevertheless, Butz's decision has been greeted with dismay in the infested states, which have been relying on federal support for their efforts to control the ant. Typical of such reaction is a letter sent by the commissioner of Alabama State Department of Agriculture to Representative William L. Dickinson. "To deprive the inhabitants of the infested states of the program which gives some relief from the Imported Fire Ant is reprehensive [sic]", the letter stated.

It is unlikely that the plea will go unheeded in Congress, but exactly what will transpire is, at this stage, highly uncertain. □

Shaded area: generally infested area (eradication treatments not in progress or planned). Hatched areas: ant eradicated, and regulations removed.





Poisons, and how to dump them

The growth of concern about the tipping of noxious and toxic wastes in the UK has taken place against a background of increasingly tough legislation, the programme for which has nonetheless now come to a temporary halt as the result of a tight economic situation. John Newell reports.

DURING the next few months, the first reports are expected to emerge from technical groups within the Department of the Environment's waste disposal section. These groups are looking into the best ways of disposing of specific toxic wastes, including arsenic compounds, acids, mercury and other heavy metals in various concentrations, tarry residues and metal-finishing wastes.

Recommendations will take into account the 1974 Control of Pollution Act, which allows stringent requirements to be laid down for the disposal of particularly hazardous wastes. As soon as the Act comes into effect, all private operators will have to apply for licences for sites used to dump all noxious wastes. The power to withhold or remove licences, and the power to prescribe special codes of practice for

especially toxic wastes provides local authorities with two powerful new safeguards. Nor do they any longer have the burden of proof of damage to the environment, as was the case under the 1972 Disposal of Toxic Wastes Act.

The 1974 Control of Pollution Act, the implementation of parts of which has been delayed for at least a year, goes further than the 1972 act in making it a duty for each local authority to make a survey of all industrial and domestic wastes created in its area. Local authorities must make detailed plans for the disposal of all wastes, and are compelled to introduce a licensing system for both landfill disposal sites and waste disposal operators. Local authorities will also be required to set out and maintain equivalent conditions for their own refuse disposal operations.

Although industry has welcomed the new act in principle, and has also supported the development of chemical treatment plants and modern incinerators, the economic situation has meant that the actual usage of such plants is somewhat disappointing, especially where smaller companies are concerned. But the implementation of the 1974 act should help those companies by ensuring that adequate disposal facilities are provided by local

authorities.

For the foreseeable future, all existing means of disposal of toxic waste are bound to continue in use. The more recently designed chemical treatment plants for toxic wastes have, however, been designed with a view to increasing substantially the small proportion of their input which is at present recycled. Re-Chem International (which build chemical treatment plant) estimates that about 1 million tons of hazardous waste are produced annually in the UK, and this is thought to be increasing by about 1% (10,000 tons) a year.

The present need to restrict local authority expenditure is inevitably delaying the timetable for bringing the act into full operation. Nonetheless, the parts of the act requiring local authorities to survey all waste, products and those concerned with the introduction of increased penalties for existing pollution offences, are being introduced over the next few months, as they require little or no extra manpower.

On the other hand, the requirement to produce a formal waste disposal plan has been deferred for a year, after discussion with local authority associations, and so also has the requirement to introduce the licensing system. The parts of the act relating to water pollution will also be implemented in phases starting next year.

The fact that the act is now on the Statute Book is, however, already causing alterations in waste disposal practice in preparation for what is to come. Landfill will remain the principle means of disposal and the Department of the Environment has had a research programme on landfill with toxic waste running since 1973. One aim is to produce a code of practice for use by waste disposal authorities in the selection and control of disposal sites. The code will be concerned with water pollution and atmospheric pollution hazards, odour nuisance, and contamination of soils and vegetation. The team involved is developing a mathematical model of the behaviour of hazardous materials in the interiors of landfill tips.

Sheer shortage of tipping space is already encouraging the recycling and reclamation of ordinary household rubbish and this will probably soon be the case for actively toxic wastes, too. The Department of the Environment points out, justifiably, that the 1974 Control of Pollution Act has been the model for an EEC policy directive and that UK legislation in this field is ahead of most of the world. But the pressure in the future seems bound to be directed towards more incineration, more chemical treatment to render harmless, and more active recycling of toxic wastes. □

international news

A PRESIDENTIALLY appointed commission will recommend later this month that the federal government should lift its moratorium on funding for research on living fetuses. In place of the moratorium—which was imposed by Congress last year—the commission will recommend a set of stringent controls to ensure that such research is carried out according to strict ethical guidelines.

The commission's recommendations, which were agreed at a public meeting late last month, will be sent to the Secretary of Health, Education and Welfare along with a report providing justification for them. The secretary must either use the recommendations to draft regulations governing federal support of foetal research, or state publicly why he cannot accept them.

The commission—the National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research was established by Congress last year and was given four months in which to draft regulations governing foetal research and two years to draw up recommendations governing other types of human experimentation. The recommendations are, however, far from being the final word on the

Foetal research ban to end

by Colin Norman, Washington

matter, because foetal research has unfortunately become ensnared in the loud and bitter dispute over abortion which has been raging in the United States for the past couple of years. The anti-abortionists are unlikely to accept the commission's recommendation.

The commission decided, in short, that therapeutic research, designed to benefit either the foetus or the mother, should be actively encouraged by the federal government, but that all foetal studies should be subjected to review procedures at the local level. One review committee would ensure that the study is ethically acceptable and likely to produce worthwhile results, while another would monitor the experiment to ensure that it is conducted according to ethical guidelines. The mother's consent, and if possible that of the father, would also be required before any research on living fetuses could be conducted.

The two most controversial areas of

foetal research—those involving studies of foetuses which have been scheduled for abortion but which are still in the womb, and experiments on living, but non-viable foetuses after they have been aborted—would require prior review by a national ethical review board, the commission suggests. Examples of the first category are the administration of drugs to pregnant women about to undergo abortion, and subsequent examination of the foetal tissue to see whether the drug crossed the placenta. Four researchers are, in fact, awaiting trial in Boston on charges arising from such an experiment. And the second type of research includes, for example, studies of foetal circulation.

Although the commission's recommendations are likely to play a vital part in the development of policies governing federal support of foetal research, they have already been made largely redundant in some parts of the United States. Biomedical researchers conducting some types of foetal studies in those places face stiff prison terms, and so for them the question of whether or not the federal government would support their research is entirely academic. □

ALTHOUGH it is common practice for elders of the scientific community to lament the decline in federal support for science, and to warn that the fabric of scientific research will be irreparably damaged unless the government adopts less parsimonious policies, a recent speech along those lines by Philip Handler, the President of the National Academy of Sciences, is drawing considerable attention.

Delivering his annual report to the academy last month, Handler noted that "there are scattered clouds on the science horizon" which include lack of growth in the science budget, threats to the peer-review system by which grants for basic research are awarded, and a "bookburning" attitude in Congress.

Proceeding from the observation that the Ford Administration's budget for next year is essentially a "no growth" budget for basic research, Handler drew attention to the fact that "there are continuing pressures to maximize the use of available federal resources for the support and conduct of that science which promises earliest applicability". In particular, he suggested

that "support of basic research by the Defense Department becomes increasingly constrained", and "emphasis on specific applied programs using funds formerly available for free research is a matter of policy at the National Institutes of Health, particularly the National Cancer Institute".

Those complaints are standard fare, but Handler suggested that "other factors loom as more worrisome", and "one can see in the offing a powerful threat to the peer-review system for decision making with respect to selection of research projects for support". The threat comes from the recent spate of press releases, emanating chiefly from the office of Senator William Proxmire, which have poured ridicule on science projects with trivial or funny-sounding titles. Such tactics, which are designed chiefly to generate cheap publicity, have planted in the public's mind "the seed of distrust in the judgement-making apparatus", Handler suggested.

Such distrust caused the House of Representatives last month to pass a preposterous amendment to the author-

isation bill for the National Science Foundation to give Congress veto power over every individual grant awarded by NSF. Although the Senate is unlikely to follow suit, Handler noted that "the point is that the House came dangerously close to passing an amendment which was tantamount to book-burning". He continued, "the intrusion of political considerations in the award of grants by an agency whose principal purpose is to support the continuing dispassionate search for truth and to support education in that knowledge which has been gained by rigorous investigation according to the highest canons of our disciplines, is unacceptable". The House's action, Handler said, "is a giant leap backward by a supposedly egalitarian House which adopted a procedure appropriate only to authoritarian regimes".

Handler urged the scientific community to come to the defence of research projects that are subjected to attacks from Senators and Congressmen in search of publicity because if such projects "are not defended by all of us, it will be our turn next".

Soviet rocket puts India in space club

by Narender K. Sehgal, Jullundur

FOR the second time in less than twelve months India has forced her way in to an exclusive club, some of whose members happen to believe that she has no business being there in view of her poverty and food problems. First, it was the nuclear club on May 18 last year and then, on April 19, 1975, India's first ever satellite was successfully launched on a Soviet Inter-cosmos rocket—making her the eleventh member of the space club.

Prominent members of the scientific community have expressed happiness at the accomplishment. "This is another proud day for all Indians", said Dr M. S. Swaminathan, President of the Indian Science Congress Association, in Calcutta. Nearly everyone, while expressing satisfaction, said they were looking forward to the day when an Indian satellite would be launched from within India itself.

Named Aryabhata, after the famous Indian astronomer-mathematician of the fifth century AD, the blue and violet, 26-faced, diamond-shaped satellite weighs 360 kg and is orbiting the Earth every 96.41 minutes at an altitude varying between 564 and 623 kilometres and an inclination to the equator of 50.4°. The operational life of Aryabhata is estimated to be about six months, because that is how long the stock on board of an inert gas (used in the spin-up stabilisation mechanism of the satellite) will last. But the satellite is expected to stay up in orbit for about 2½ years. Aryabhata is equipped to carry out three scientific experiments during its operational life. One experiment, designed by ISRO (Indian Space Research Organisation) scientists themselves, will make X-ray

measurements; another designed by the Tata Institute of Fundamental Research, aims at detecting high energy neutrons and rays at times of intense solar activity; and the third experiment, set up by the Physical Research Laboratory at Ahmedabad, will study electrons in the ionosphere and ultraviolet radiation in the night sky.

Work on Aryabhata began following an agreement between ISRO and the USSR Academy of Sciences in May 1972. (The USSR help was sought only for the launch.) The Indian Scientific Satellite Project, established by ISRO in December 1972 at Peenya near Bangalore, was given the overall responsibility for fabrication of the satellite. But the final product was the result of a collective effort by nine major institutions, including national laboratories, private and public sector undertakings and a number of small scale industries. The flight model was designed and fabricated indigenously and tested extensively both at Peenya and in the Soviet Union. Only certain components—the solar cells, chemical storage batteries, sophisticated on-board tape recorders and spin-up gas bottles—had to be obtained from the Soviet Union. Some 'space-qualified' electronic components, too, had to be imported from abroad because they were not available in India. The laboratories at Peenya included the space simulation chambers for testing the various sub-systems of the satellite. The chambers were designed and built by ISRO engineers in collaboration with the Bhabha Atomic Research Centre at Bombay and the Electronics Corporation of India Ltd at Hyderabad. The design and fabrication of instruments for commanding the satellite from ground stations at Sriharikota in Andhra Pradesh and Bears Lake near Moscow were also carried out by Indian scientists and engineers. The entire Aryabhata project cost Rs 4.3

crores (= Rs 43 million), including the infrastructure and facilities that went into creating it, and was executed in 30 months.

With this feat, the inevitable cropped up again. Among the first questions that the Prime Minister Shrimati Indira Gandhi and the Chairman of the Space Commission, Professor Satish Dhawan, had to face were variants of the following: "Why has India chosen to spend money on a space programme? How does Aryabhata fit in with the country's development priorities? Will there be any returns from these investments? Will they justify the monies and efforts expended on them?"

Fortunately these questions did not seem as difficult to answer as the ones posed about the nuclear explosion last year. For one thing, the objectives of Aryabhata are certainly not as controversial. Second, India has already been making use of foreign satellites, among other things, for communications. Moreover, a major educational experiment SITE (Satellite Instructional Television Experiment), to be launched in July this year, will make use of the American ATS-6 satellite for a year to beam television programmes in regional languages to receivers in several thousand villages in selected states.

Scientific and technological self-reliance is the under-lying theme behind the space programme, as is also the case with India's nuclear effort. The Indian government happens to believe that the nation's economic and social well-being will be helped by keeping abreast of the latest advances and developments in various scientific and technological fields. India, with the third largest scientific and technological personnel force in the world next only to the USA and the USSR, intends to make fuller use of space technology in the fields of communications, television, meteorological studies, earth resources, agricultural crop surveys and so on. Specifically, Aryabhata is designed as India's first step towards establishing her own capability in the area of satellite fabrication. The three scientific experiments, though important and "most modern" in the words of Academician Boris Petrov (Chairman of the "Intercosmos", the Soviet organisation in charge of international cooperation), are only of secondary importance; the mission would be considered successful if all systems aboard Aryabhata function normally, irrespective of the experiments. Happily, first indications are that all instruments and equipment aboard the satellite are working normally and well.

As a next step, Aryabhata 2 may be launched soon. It will carry television camera systems to survey mineral

THE Swedish and Russian Academies of Science have concluded an agreement on exchange of researchers and research. The agreement is unique in that, unlike the others the Russians have with western countries, it allows for the host country to invite specific scientists by name for study visits rather than accepting the other country's nominations. Each country will have 60 man months a year of expertise from the other, and the main areas of exchange are expected to be biology, physics and chemistry.

The agreement formalises and broadens already existing cooperation between Russian and Swedish scientists. Professor Hans Vilhelmsson at the Chalmers Institute of Technology,

for example, has already had fruitful exchanges with Russian researchers in plasma physics. Last year he invited four specific Russians to a workshop at Chalmers. All came, and the meeting produced reports to be published soon in *Physica Scripta*.

Upholding the individual invitation clause will of course depend on goodwill between the scientific communities in each country and also on the Soviet government. There is plenty of goodwill at the moment, and an obvious incentive for the Russians at least to maintain theirs. For who knows what visiting Russian scientist may impress his Swedish hosts so much that they award him a Nobel prize.

—Wendy Barnaby

THE new Soviet icebreaker *Arktika* has received, throughout her construction, the continuing press coverage reserved for major Soviet prestige achievements—interviews with the designers, scientists and research teams and a number of extensive, but often peripheral, articles related to the history of nuclear-powered icebreakers. To enhance this aura of prestige, the commissioning date of the *Arktika* was apparently chosen with an eye to symbolism: the hammer-and-sickle was hoisted on the new flagship of the icebreaker fleet “on the eve of May Day and on the threshold of the thirtieth anniversary of the victory over Nazi Germany”.

Yet the *Arktika* and her sister icebreakers, which can extend the navigation season of the Northern Sea Route by several weeks each year and which undoubtedly rank as a major Soviet achievement, may well mark the quiet demise of an even more spectacular achievement *in spe*—the diversion of the Siberian rivers. This concept of a super-irrigation scheme, by which the waters of the Ob’ and Yenisei would be “turned backwards” (conveyed by a series of canals to irrigate the deserts of Soviet Central Asia and, by way of the Volga, to replenish the shrinking Caspian) has been a dream of the Soviet planners for many years. The waters of the Ob’ and Yenisei would no longer spill wastefully into the Arctic Ocean, but would have their directions of flow reversed, to the bene-

fit of Soviet agriculture and the Soviet economy.

But now, likewise in the name of the Soviet economy, the construction of an icebreaker fleet implies another use for these waters. The home port of the fleet, Murmansk, is normally ice-free throughout the year. Some 1,500 miles eastward, however, lies the new mineralogical centre of Noril’sk, producing rich ores of non-ferrous metals. The only practical outlet for the products of Noril’sk, and the only means of pro-

Ice on troubled waters

from Vera Rich, London

viding its 150,000 inhabitants with essential supplies, is the rail link to the port of Dudinka on the Yenisei, and thence by river and across the Kara and Barents seas.

This river and sea route is normally blocked by ice for seven or eight months of the year. During these months, large icebreakers, such as the *Ermak*, the *Lenin* and now the *Arktika* must force a way through the ice for the freighter convoys. But these large ships (the *Arktika* is 140 m long and 30 m wide) can only operate between Murmansk and Dikson at the mouth of the Yenisei gulf. To conduct the convoys on the river route to Dudinka, “medium power” icebreakers (presumably with conventional engines)

must be used.

If the Yenisei diversion scheme is still at the forefront of Soviet development plans, it would seem logical to assume that these smaller icebreakers are an interim measure and that, in the fairly near future, they will be replaced by a rail link from Dudinka to Dikson (which could, itself, rightly be considered a prestige achievement). This, however, does not seem to be the case. According to a *Novosti* release of March 10, “new up-to-date icebreakers with a small draught and able to negotiate the Yenisei are under construction”. Although it is difficult to project the expected lifetime of these small-draught vessels, taking into account considerations of cost-effectiveness and the time still needed for construction, it would seem that the planners of the Northern Sea Route envisage that the lower reaches of the Yenisei will remain “navigable” (at least to icebreakers) until the end of the century. Which poses the problem: what becomes of the diversion scheme? Has it been quietly relegated into the sphere of plans that are desirable but not, within the foreseeable future, feasible (although still, from time to time, receiving the occasional tribute of press coverage?) or is there some lack of communication between the two planning bodies concerned—a division of interests, each demanding the use of the waters of the Yenisei in the service of the Soviet economy but pulling, literally, in opposite directions?

deposits and agricultural crops. The satellite—actually a back-up model for the one now in orbit—is reported to be almost ready. Encouraged by the success of its maiden venture, ISRO intends to begin negotiations soon with the USSR for a fresh agreement to launch it.

Satellite fabrication is only half the story however, as far as self-reliance is concerned. Work has also been in progress on a four-stage satellite launch vehicle at the Thumba rocket station in Kerala. But because of a shortage of funds, the project is running several years behind schedule. Moreover, it will only be able to launch a small payload weighing no more than 40 kg to start with—a mere ninth of the weight of *Aryabhata*.

It is just as well that the first Indian satellite was not launched from Indian soil, for possession of a rocket powerful enough to launch a satellite hundreds of kilometres into space would have most certainly been seen by critics aboard as an effort to develop an intermediate range ballistic missile capability. But here in India, one can discern no indication of any such attempt,

if one goes by the small sums of money earmarked for the space and nuclear programmes—and the fact that the military has been kept well away from these programmes, almost to the extent of deliberate exclusion.

Notwithstanding hostile criticism abroad, there are many in India who believe she should keep all her options open, nuclear weapons and all, even if she does not intend to use them. A technical commentator for a national daily newspaper recently deplored the almost total lack of coordination between the nuclear and space programmes, on the one hand, and the military establishment on the other. He concluded thus: “Viewed from nearby and even with the bias natural to any one with the country’s interest at heart, we are far from a nuclear weapons capability. Both the atomic energy and space research budgets are pegged at low levels. They cannot sustain a military capability in nuclear weapons. However, research projects must not, by deliberate choice, exclude the full range of applications of research achievements. Since both atomic energy and space research programmes

are doing so, at least in the military field, Indian planning could do with some redirection, even at the risk of inviting further criticism overseas.” □

US university R and D

THE US National Science Foundation has published a set of figures chronicling the decline in federal support for research and development in universities in the United States. NSF’s figures show that total spending on research and development in higher education increased from \$2,900 million in 1973 to about \$3,000 million in 1974 which, when inflation is taken into account, represents a drop of about 5% in purchasing power. Within that total, however, federal support declined by 8% in constant dollars. Part of the decrease is explained by a book-keeping change involved in the transfer of the Draper Laboratory from the Massachusetts Institute of Technology into the independent non-profit sector, but even taking that into account, NSF points out that the purchasing power of expenditures on academic R & D declined by 3% and federal support declined by 6%. □

CANADA and the USA share in the ratio of about 2:3 the most extensive and one of the richest bodies of fresh-water in the world—the Great Lakes system which consists of five lakes with a total surface area of 94,250 square miles. Not only are the lakes themselves large and valuable but they are surrounded by a region of gentle topography, temperate climate, vast mineral resources and bountiful farmland. Through the St Lawrence Seaway, opened in 1959, the lakes serve as a direct water link with the rest of the world, bringing ocean shipping to the heart of North America. As a result the system forms the hub of the North American population, with nearly 35 million people living in or near the Great Lakes Basin (one in every three Canadians, and one in every eight US citizens); predictions for the end of the century suggest a doubling of these figures.

When they were first discovered the vastness of the Great Lakes made them seem indestructible compared with the works of man. No longer. Their increased use, and rising industrialisation and population around their shores, makes the lakes system seem fragile in the face of man's multifarious interventions. Indeed they have proved so. Lake Erie was declared eutrophic some years ago. Both Lakes Michigan and Ontario have ceased to be safe for swimming and both have suffered serious algal blooms and fish-kills. Yet there is still a need to stretch the lakes' capacity to meet a swelling demand from the human population.

With the specific object of improving management of the Great Lakes as a whole to meet this demand, Dr D. V. Anderson, of the University of Toronto, proposed an International Field Year for the Great Lakes (IFYGL) as North America's principal contribution to the International Hydrological Decade which supposedly ends in 1975. Lake Ontario was chosen for this intensive study, because it is the smallest of the five (7,340 square miles) yet typical of the others (excepting Erie), convenient to study, situated in a growing population area and already showing signs of deterioration. The concept was that most of what was learned from the intensive study of Lake Ontario would be applicable to the other Great Lakes and even to other large lakes throughout the world. The project was delayed because of difficulties encountered in the USA, first in finding a 'lead' agency and then in obtaining the money for research. (Canada, with its centralised national research council structure and funding, was readily able to provide

support by simply adding to ongoing projects.) The budget of \$35 million called for was eventually collected and the IFYGL was initiated in April 1972.

Curiously there had been no co-operative agreement between the USA and Canada on protective and restorative measures until that date. In the rider to the agreement then made, the point was made that although the technology for repair was adequate, solutions to many of the problems could not be prescribed because knowledge and understanding of the various interactions within the systems are "woe-

Great Lakes report

from Angela Croome

fully inadequate". Before the Field Year there had been plenty of individual scientific projects going on but there was no overall view taken. The six 'core' programmes selected—lake meteorology, energy balance, terrestrial water balance, lake water movement, biology and water chemistry—were intended to provide the data for sound solutions and wise future management. The publication of the preliminary batch of reports provides some opportunity to judge how successful this has been. Water quantity and water quality lie at the root of all the problems of the Great Lakes. The first shows up in such problems as flooding, eroding shorelines, low water in ship canals, storm damage to coastal facilities and cutbacks in production at the numerous hydroelectric plants. There is, in addition, the ice nuisance which both precludes inter-lake navigation in winter and often severely restricts the flow of water to the hydroelectric power stations. It has been suggested that waste heat from the increasing number of thermal electric stations be used to melt the ice, but this is a step to be taken only after weighing up all the other factors. Problems with water quality include sewage pollution, safe water supplies and, particularly important for Lake Ontario, the collapse of commercial fisheries. The unforeseen migration of alien species such as 'alewives' and parasitic lampreys from the Atlantic up the navigation canal network has played havoc with favoured game fish though overfishing met this menace half-way.

As it happened spring 1972 to spring 1973 was atypical in one respect: the weather. It was the wettest 12 months for years. Moreover on the basis of 30 years of records the driest month of the year is usually June but in the Field Year it was the wettest (by a factor of nearly two). Not surprisingly,

the inflow and outflow to the lake was also abnormal, with the July runoff four times higher than usual. Whereas pathways and balance could be studied, the normal hydrological cycle was hardly manifest. The arrival of Hurricane Agnes during some days in June however, provided a bonus in the form of a thorough check of the reliability of rainfall radars. It was found out that the radars underestimated the volume of rain by as much as 40% and the errors were particularly marked when the heaviest rain occurred. Inadequate warning of floods is attributed to the radar errors.

A special study of the lake-atmosphere boundary layer by several means was incorporated in the programme partly because of the increasing interest this interface is attracting and partly because Lake Ontario offered a convenient site on almost a 'sea' scale. Preliminary results indicate that a large fraction of the annual evaporation occurs in a very few intense, cold-air outbreaks in autumn and that on these occasions there is an extremely large gradient of evaporation across the lake. A further curiosity identified by this programme was the occurrence from time to time of a wave-driven wind on the lake. (Some wave model studies carried out in 1966 had suggested that such a phenomenon might exist.) Specific studies of airborne sulphur dioxide contamination blowing in near Niagara (calculated to amount to several tons a year) showed that a lake with a pH near 8 may act as a very effective SO₂ 'sink' for the atmosphere.

The study of fish population, which is of major significance to the Canadian authorities as they are in the midst of attempting to restore the ravaged Lake Ontario fish stocks, was specifically aimed to be a first step in a continuing programme to guide lake management. Collections of fish were made over the whole lake in all seasons except winter. A total of 63 species was collected and produced few surprises. Samples did, however, include a few deep water sculpin (thought to have been extinct) but no examples of the equally popular indigenous cisco. Introductions, the American smelt and the alewife, dominated the main trench and were also common pelagically. The authors end their paper with a heartfelt comment that "ecology is poorly understood in the physical aquatic sciences". This work is now a continuing 'core' programme for the Canadians, who hope eventually to defeat the invading lamprey and return their lake to full health and fish production.

THE constantly growing world shortage of non-human primates for research laboratories, which has already held up some Israeli immunological and pharmacological studies, will apparently lead to the development of a new branch in the economy of several kibbutzim—the raising of monkeys on a commercial basis. Such a scheme has been in the air since the early 1970's when the National Council for Research and Development sponsored a feasibility study. But it was rejected three years ago because it was still cheaper at that time to import non-human primates from countries in Latin America, Asia and Africa (where they only had to be trapped) than to raise them in captivity, in itself a risky venture.

The cost of monkeys on the world market has, however, significantly increased in the interval and, more important, they are not always readily available even for buyers willing to pay the ruling prices. At the same time, a number of kibbutzim have succeeded, without fanfare or official sponsorship, in raising such animals on a modest scale. Local collective settlements originally brought in monkeys for their special educational farms, where kibbutz children cultivate crops and raise animals on their own, with a minimum of adult supervision. Most of these primates adjusted well to kibbutz life and in some cases even multiplied.

Thus it was natural for Israeli primatologists to view the kibbutzniks as potential partners in a scheme to raise non-human primates on a larger scale for local laboratories and perhaps for export. Initial plans call for the creation of several pilot centres, in places ranging from the relatively cool Galilee to the sometimes very hot Negev. Their first inhabitants will be rhesus, vervet and squirrel monkeys, the progeny of which, if all goes well, will be available for marketing in 1978.

It will be a long time before the kibbutzim can produce enough monkeys to meet the requirements of local laboratories (about 500 animals a year at present), let alone have a surplus to sell overseas. But if the scheme proves successful, it will be of great benefit to research scientists here as well as a welcome source of income for the kibbutzim, particularly since kibbutz children will do most of the routine work that does not involve physical contact with the animals.

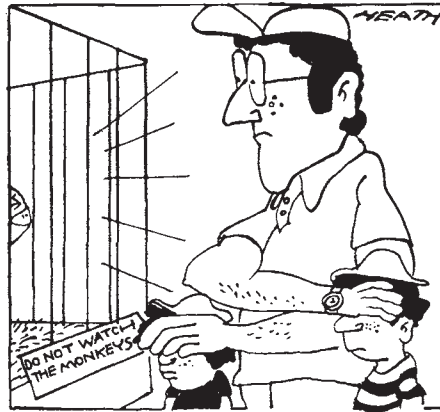
There is however, still some opposition to be overcome at the settlements themselves. One kibbutz teacher, for example, is concerned with the possibility that raising the primates will create educational problems. "It is well known that many monkeys in captivity spend a great deal of time masturbating

in their enclosures. How", he asked me in great bewilderment, "am I going to explain that to my first graders?"

● Links exist between Israel's kibbutzim and her institutions of research and higher learning on many levels. Blessed with a highly intelligent population, the settlements are quick to use research results relevant to their industrial and agricultural branches, as well as to their educational system. Moreover, some 2,000 university students and almost 100 university faculty members are from kibbutzim.

Letter from Israel

from Nechemia Meyers, Rehovot



Although few participants were aware of the fact, the man who organised this month's EMBO Workshop on muscle cell culture in the study of gene expression during differentiation, Professor David Yaffe, of the Weizman Institute is a member of Kibbutz Givat Brenner. This same collective settlement boasts yet another Professor at the institute, Achi Brandt, the mathematician. Head of the Pure Mathematics Department, Professor Brandt contributes not only his salary but also his know-how to the kibbutz, helping it to use computers in planning the most efficient exploitation of its manpower and production facilities.

As Brandt recently explained it: "To establish the best method of distributing kibbutz resources, you must first assess the possible economic activities, their income and cost, and the availability of such components as manpower, land, capital and water. At the same time, you cannot overlook tricky agro-technical, seasonal and even ideological restrictions (such as the desire of most kibbutzim to minimise or, if possible, eliminate the 'exploitation' of hired labour in kibbutz fields and factories). With these data, the optimal plan can be found by a properly designed computer program which can also tell us the relative importance of each restriction imposed on the system."

Brandt even showed his fellow kib-

butzniks how a computer could be used to help the settlement reach decisions on social issues. "For instance", he told me, "we have long had a fierce dispute as to whether our children—who now all sleep in what we call children's houses—should not perhaps sleep at home with their parents. So I devised a questionnaire to sift out actual needs from mere opinions, and wrote a program which analysed the answers. We discovered that, quite contrary to what we thought, only a minority of kibbutz parents really feel that their children should sleep at home. At the same time, this minority wants to change very badly, so that some modification in the system may very well occur at Givat Brenner as it has at other settlements."

● The unique ethnic character of Israeli kibbutzim has long attracted the interest not only of the sociologists and anthropologists but also of medical researchers, who welcome the opportunity to measure the positive and negative effects of life in Israel on the health standards of various communities. Some years ago they discovered for example, a clear link between the onset of Westernisation—particularly the adoption of Western eating habits—and an increase in the incidence of heart disease and diabetes among Jews from Yemen, who, before their emigration, had lived in a 14th century society and subsisted on a virtually fatless and sugarless diet. As they began eating more fats and sugars, they grew increasingly prone to heart disease. Of course one shouldn't overlook the fact that their life expectancy here is still much higher than it had been in Yemen, where modern medical care was unobtainable.

Now the same pattern has developed among the Bedouin. Doctors at the Negev Centre Hospital in Beersheba, where hundreds of Bedouin are treated each year, report a steady increase in heart disease among them, even though it still remains much lower than among the Jewish residents of the area. But, as with the Yemenites, overall life expectancy has risen to West European standards (70+ for males), by comparison, for example, with 50 years in neighbouring Egypt.

Israel's best known ethnic malady is Tay-Sachs Disease, a fatal genetic disorder limited almost entirely to infants whose forebears came here from certain parts of East Europe. Although the disease is still incurable, Israel—like Jewish centres in the USA and Canada—has instituted a Tay-Sachs screening and counselling programme to advise potential parents whose offspring might be affected.

Jews from Oriental lands also have their 'own diseases', one of which—favism—was responsible for a com-

pletely unnecessary mini-panic here recently. The hereditary disease strikes people who lack a particular enzyme (G6PD), one which protects red blood cells against the noxious effects of the fava bean and other materials, and thus prevents anaemia. It is by no means limited to Jews, as it affects, among others, Scandinavians, American Negroes and groups in South-east Asia. But among Jews it is limited almost exclusively to people who have lived in Iraq, Kurdistan or Iran.

In spite of this well established fact, the Ministry of Health last week dramatically announced that all Israelis should refrain from eating fava beans (which, together with common medi-

cines like sulpha drugs and aspirin, trigger favism). The sale of fava beans diminished, of course, but rose again when citizens were informed that unless they came from those three countries they could eat the lentils to their heart's content. A screening programme now being carried out at local hospitals should clarify the problem for all Israelis in the course of the next few years.

● The dangers of inbreeding within a particular ethnic group are starkly emphasised by the problems facing the Samaritans, an ancient Holy Land sect which now numbers some 450, half of whom live in Holon, a suburb of Tel Aviv, while the other half live in the

West Bank town of Nablus. Because 58% of Samaritan marriages take place between members of the same family, an abnormal number of physically or mentally handicapped children is born to members of the community. New blood in the form of five Jewish brides has improved the genetic mix in recent years, but the community still remains dangerously inbred.

Yet these people, descendants of the Good Samaritan, show an extraordinary will to survive. They now number more than three times as many as they did a hundred years ago, when *The Times* reported that there were only 135 Samaritans in the entire country. □

correspondence

Mediterranean mercury

SIR,—The article by Alexander Dorozynski (April 17) on the "Mediterranean poison fish forecast" made by Dr Maurice Aubert, Director of the Centre d'Etudes et de Recherches des Biologie et Oceanographie Médicale in Nice states that more than 1 p.p.m. of methylmercury is present in nine species of fish caught in the Mediterranean. There is a calculation attributed to Dr Aubert worth quoting: "Since it has been found that only 4 to 5% of mercury absorbed is fixed in the organism, the weekly absorption of food containing 2 mg of mercury will result in a fixation of 80 µg. At this rate the lethal dose is reached about 20 years". The lethal dose according to Dr Aubert is 80 mg, which is reached in the fisherman by the following equation:

$$2 \text{ mg} \times 0.04 \times 52 \times 20$$

This calculation has no scientific basis. First Japanese data indicate that 80 mg is the lowest toxic body burden and not the lethal dose. Second abundant data reviewed by expert committees show that approximately 95% of the ingested methylmercury is absorbed from the gastrointestinal tract and that the average half life of methylmercury in the human body is 70 days. This means that the body burden of the fisherman in question will be 24 mg after 6 months, 28 mg after 1 year and 28.86 mg in the steady state when he will excrete exactly 2 mg of mercury a week.

The same criticism applies to Dr Aubert's calculation for the child victims of Minamata. He also misquotes the lowest level of hair mercury associ-

ated with intoxication. According to the work of the Swedish Expert Group on the Minamata epidemic, 60 µg/g Hg signals the danger of intoxication rather than twice the level (7.39 µg/g) found by Dr Aubert in a fisherman. Based on the relationship between the concentration of mercury in hair, the blood level, the body burden and the weekly dose, his fishermen probably had a body burden of less than 10 mg, which assuming steady state conditions was the result of the weekly consumption of 0.7 mg mercury as methylmercury.

Yours sincerely,

L. MAGOS

MRC Laboratories,
Carshalton, UK

In line 9 of paragraph 5 of the article, 25 µg should read 25 mg.

Plants

SIR,—Rain forests, vanishing wild life and the destruction of rare wild flowers by reservoirs are all sure of space in periodicals and funds for conservation on grounds of preserving the gene pool, yet garden varieties of vegetables are in greater danger of extermination. The FAO are now searching Turkey for hardy varieties still grown by peasants, but Europe is destroying her genetic heritage from the best of motives.

Modern methods of seed storage make it possible to keep packets "in mothballs" for as long as 200 years and I suggest that stocks of all deleted varieties should be donated to a National Seed Library, ideally sited at a University such as Reading, from which they would be available to plant breeders and enthusiasts. We would be glad to hear from those who are

seriously interested in this project and from Foundations and others who would make grants to start a World Vegetable Fund, like the World Wildlife Fund, but more urgent in view of the greater pace of extermination.

Yours sincerely,

LAWRENCE D. HILLS

Henry Doubleday Research
Association,
Braintree, Essex, UK

Methinks he doth edit too much

SIR,—Every new scientific journal to appear sports a long list of associate editors, members of the editorial board, advisory editors or whatever. These are generally academics who are presumed to guarantee the quality of the product; my suspicion is that they rarely do anything beyond collect an annual fee, referee a paper or two and line their shelves with complimentary copies of the journal which they don't read. One journal that I see has an advisory board of six in addition to twenty or thirty associate editors; all the advisors have a Nobel Prize but only one in a field close to that of the journal.

I don't begrudge a fellow one sinecure, but it is my impression that some hold five or ten editorial positions. How about a prize for the man with the most appearances?

Yours faithfully,

M. CHARLES

Boston, Mass

A year's free subscription for he or she whose nominee tops the list—but nothing for the nominee, whose shelves are probably full already. Entries by July 1—Ed.

news and views

Two very beautiful papers on insulin appeared in *Scientia Sinica* in December 1974 (17, 752 and 779). They continue a sequence of scientific publications which began in 1961 and includes the total synthesis of sheep insulin, described in 1965. They are published by groups of research workers in China: in Peking in the University (Departments of Biochemistry and Organic Chemistry) and in the Institute of Physics of the Academia Sinica, in Shanghai in the Institutes of Organic Chemistry, Biochemistry and, most recently, Zoology. They provide impressive evidence of integrated researches in different disciplines and laboratories.

The first of the present two papers describes the derivation of an electron density map of insulin in rhombohedral 2-Zinc insulin crystals at 1.80 Å resolution. It gives useful experimental details of the preparation of the crystals and heavy atom derivatives, of the measurement and interpretation of the X-ray diffraction data and of the stages of solution of the structure. The model and electron density map (clearly of very good quality) are illustrated in colour and the atomic arrangement is described in detail. Ramachandran plots and a plot of the distribution of C γ side chain atoms viewed along C α -C β illustrate some of the stereochemistry observed.

The structure is essentially the same as that found in parallel crystallographic studies in Oxford; indeed, in 1972, in Peking, we compared in detail our earlier electron density maps, both drawn at a scale of 1 cm to 1 Å. There was actually a moment of acute concern when we put the maps together first and they did not fit—until we realised one was upside down compared with the other. We then saw that there was very good correspondence, if not exact agreement, between them. Correspondingly now, in the insulin

Chinese work on insulin

from Dorothy Crowfoot Hodgkin

dimer, molecule I Peking is molecule II Oxford, and molecule I Oxford is molecule II Peking.

One might not wish, in all cases, to see complete duplication of the X-ray analysis of a protein molecule—so much work is involved. But there are great gains in the present case from having two views of the insulin crystal structure. First, insulin is such an odd molecule and the situation in the crystal is very curious. The two insulin molecules in the asymmetric unit are very similar to one another, yet appear to differ in conformation in a number of details; it is comforting to see how closely the molecules described in the Chinese papers agree with Oxford findings. Earlier small differences between us are diminishing; it may well be that the final comparison will give us reliable estimates of the errors we must expect in general in placing atoms from X-ray data on proteins. The present Peking map at 1.80 Å resolution is the most accurate map available of the insulin electron density defined by experimental, isomorphous phase angles—and may well remain so. The calculations we are carrying out at Oxford, designed to show the electron density at 1.50 Å resolution, depend on the extension of the phases from 1.9 to 1.5 Å by various computing procedures, some very experimental. The total cross checking of our operations by the Peking analysis when we come to compare atomic coordinates should be very valuable.

Many of the comments made by the

Chinese insulin research group on their findings are similar to ones we have made ourselves, but they often add illuminating details and occasionally recognise quite different features—for example, they realised that the dihedral angles of the glycine residues, B8, B20 and B23, were all in the region allowed for D residues. This led directly to experiments by the Shanghai chemists and biochemists who showed that B23 might be replaced by D-alanine in the insulin sequence with only partial loss of biological activity, whereas activity vanishes when B23 is replaced by L-alanine.

The second paper carries a stage further the study of structure-activity relations in the insulin field through direct measurements of the interaction of insulin and insulin analogues with receptor proteins in liver and fat cells. It, too, parallels and extends work in other laboratories.

Soluble receptor protein preparations were made by the method of Cuatrecasas, of fat cells by a new method avoiding collagenase. It was found that despentapeptide insulin had almost the same binding capacity as insulin, while deshexapeptide insulin and the corresponding molecule with B23 replaced by D-alanine had reduced, but definite, binding capacity. The results strengthen the view that the non-polar residues of the B chain surface, B12, B16, B24 and B25, are important in receptor binding and that the loop B20-B23 and perhaps also the salt bridge A21-B22 assist in stabilising the conformation in this region. There is a nice discussion in the first paper of the part that conformational variability may play in assisting membrane binding in this part of the insulin molecule.

It will be splendid if we can some day soon all meet and talk over the very interesting observations that are accumulating, East and West, on the structure and function of insulin.

Revising the Cainozoic polarity record

from D. H. Tarling

Now that the main plate tectonic band wagon has passed, the dust can be brushed off the marine magnetic anomalies that were so effective in getting it rolling. It is now possible to examine in more detail the record they provide of past geomagnetic changes and to

refine the time scale of polarity changes.

The polarity time scale of Heirtzler *et al.* (*J. geophys. Res.*, **73**, 2119; 1968), based on anomalies recorded on profile V-20 SA in the South Atlantic, has stood up remarkably well. Profile V-20

SA was chosen as the longest available with no marked evidence of changes in the rate or direction of spreading, and the anomalies were dated by assuming that the observed spreading rate at the ridge axis—1.9 cm yr⁻¹—had remained unchanged for the past

80 Myr. The fact that modifications are only now becoming essential is remarkable, and the choice was, to say the least, inspired.

The first evidence of inaccuracies began to appear in 1971, as a result of discrepancies between the ages of fossil-dated sediments lying over igneous rocks which carried the magnetic anomalies and of observed differences of curvature on fracture zones in the South Atlantic (Le Pichon and Hayes, *J. geophys. Res.*, **76**, 6283; 1971), which indicated two-stage spreading (see Mascle and Sibuet, *Nature*, **252**, 464; 1974). At that time the evidence was taken mainly to confirm the available scale, as the differences were generally less than 5%, which were insignificant for the analyses then being carried out.

Since then, however, detailed studies of the anomaly patterns on fast spreading ridges, such as those described by Rea and Blakely on page 126 of this issue, combined with the polarity record in deep-sea sediments, such as that reviewed by Opdyke (*Rev. Geophys. Space Phys.*, **10**, 213; 1972), have provided a generally acceptable revision of polarity scales for the past 22 Myr. The older parts of the scale, however, are not so amenable to checking. Palaeontological dating of sediments lying immediately over igneous rocks which carry the anomaly pattern are uncertain because of the lack of precision in the radiometric dating of stratigraphic zonations of the Tertiary. This arises because most available dates are based on measurements of potassium and argon isotope ratios in glauconites within stratigraphically dated

sediments. During the burial of sediments glauconite grows from illite, a mineral which often contains appreciable amounts of relic argon; and further burial may also be responsible for later loss of argon from the glauconite. Furthermore, most deep-sea drilling stopped on encountering the first igneous rocks, some of which are intrusive and therefore post-date the sediments and others of which could be more extensive, isolated flows that may have flowed out over the sedimentary deposits of a few million years. Nonetheless, on such studies, Sclater *et al.* (*Initial Reports of the Deep Sea Drilling Project*, **22**, 381; 1974) have suggested modifications to the ages of certain anomalies; in particular, anomaly 24, which is 60 Myr old on the original scale of Heirtzler *et al.*, is tentatively redated at 56.5 Myr.

Anomaly 24, the oldest found in the North Atlantic between Greenland and Europe, is one of the most critical anomalies (Vogt and Avery, *J. geophys. Res.*, **79**, 363; 1974). On the previous time scale, anomaly 24 predates the first eruptions of flood basalts in Greenland, the Faeroes and Britain, yet it seems reasonable to assume that such flood basalts would be released during tensional rifting immediately preceding continental separation, rather than after the separation has occurred. Molnar and Francheteau, on page 128 of this issue, suggest one explanation for this discrepancy.

It is possible, they reason, that anomaly 24 is younger than previously believed and that similar arguments hold for the age of anomaly 32.

Molnar and Francheteau are careful not to suggest a possible age for these anomalies because of the difficulties involved in obtaining precise radiometric ages for the flood basalts; the polarities of these basalts indicate eruptions over periods of less than 2 Myr, but the range of possible radiometric ages is large because of the presence of excess argon in some areas and the occurrence of argon loss in others. But careful segregations of the more reliable radiometric ages, and the newly available palaeontological evidence for the ages of sediments associated with flood basalts in eastern Greenland (Soper *et al.*, *J. geol. Soc. Lond.*; in the press) indicate a probable age for anomaly 24 of either 52 or 55 Myr, depending on the accepted radiometric age of the Thanetian Stage (compare 52.5 Myr by Tarling, *Principles and Applications of Palaeomagnetism*, Chapman and Hall; 1971) on the basis of preliminary palaeontological dates from deep-sea drill cores. This new evidence now means that it should be possible to produce a revised Cainozoic polarity time scale.

Such a revision will necessarily mean recalculation of the frequency and duration of polarity changes, re-evaluation of correlations between eustatic changes and rates of seafloor spreading, re-examination of the history of the breakup of the North Atlantic and other oceans, and so forth, but the fact that age discrepancies of some 5% are now becoming so important really reflects the coming-of-age of seafloor spreading as an extremely precise technique.

Hepatitis vaccine: a note of caution

from Arie J. Zuckerman

VIRAL hepatitis type B is distributed worldwide and there is a particular need for a vaccine for groups which are at an increased risk of acquiring this infection. High risk groups include surgeons, physicians, dentists, nurses, laboratory workers and other health care personnel, haemodialysis patients and their attendants (*Nature*, **235**, 130; 1972), children of mothers who become infected during pregnancy (*Nature*, **249**, 105; 1974), drug addicts, patients and others residing in large institutions, and persons living in certain tropical areas where sanitation is poor. There is also the view, not shared by many, that military personnel should be immunised against hepatitis B.

The repeated failure to passage serially hepatitis B virus in tissue culture has hampered progress towards

the development of a safe and effective vaccine. Attention has, therefore, been directed towards the use of other preparations for active immunisation against hepatitis B (*Nature*, **233**, 92; 1971). A known infective serum that contains hepatitis B virus was inactivated by heat and used as the immunogen by Krugman and associates (*J. infect. Dis.*, **122**, 432; 1970; *J. Am. med. Ass.*, **217**, 41; 1971; *New Engl. J. Med.*, **290**, 1331; 1974). The serum treated in this manner was not infective and it successfully prevented or modified hepatitis B in 69% of susceptible persons inoculated with the heated serum and challenged with the infective serum 4-8 months later. Diluted and heat-treated serum may be regarded as an 'inactivated vaccine' but it is a crude way of inducing immunity and it is un-

likely to be accepted for general use. This work, however, laid the foundations for unconventional immunogens that could be used as vaccines, and indeed 200,000 doses of one such preparation are now available for preliminary trials in man.

Coat protein

Isolated viral coat protein challenges the body's immune mechanism in the same way as the whole infectious agent and the possibility of using purified spherical 20 nm hepatitis B surface antigen particles, which are free of detectable nucleic acid, seems attractive. Such experimental immunogens have been prepared and a limited number of susceptible chimpanzees have been inoculated with encouraging results. But a variable amount of host

protein, and perhaps carbohydrate, may be complexed with hepatitis B viral protein in quantities apparently greater than those in most recognised viruses (Zuckerman and Howard, *Nature*, **246**, 445; 1973). These host proteins may include various pre-existing structures of the liver cell and may thus induce undesirable immunological reactions. Recently, it was reported that antigenic determinants related to human plasma proteins are constituents of hepatitis B surface antigen (Neurath *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, **71**, 2663; 1974). These determinants are apparently related to antigenic specificities on prealbumin, albumin, apolipoproteins C and D, and the gamma chain of immunoglobulin G. Other studies indicated that the larger polypeptides of purified surface antigen are probably adsorbed serum proteins which are necessary for the preservation of antigenic activity.

Host antigens

Demonstrable and significant levels of carbohydrate in purified fractions of the antigen have also been reported (Burrell *et al.*, *Nature new Biol.*, **243**, 260; 1973). The total carbohydrate content of purified 20–25 nm antigen particles is reported as 3.6 to 6.5%. Three glycoproteins were found as well as a glycolipid and three major phospholipids (Chairez *et al.*, *Intervirology*, **1**, 224; 1973 and **3**, 129; 1974; Steiner *et al.*, *J. Virol.*, **14**, 572; 1974). The carbohydrate may be necessary for maintaining the structure and functional integrity of the antigenic determinants or the carbohydrate itself may constitute a major antigenic determinant. The carbohydrate might have a novel haptenic specificity which is either virus-coded or virus-induced host cell-coded. Alternatively, the carbohydrate, and some lipoprotein components, might simply be derived from the host cell membranes as the mature virus particles are released. There may be some similarity between such a carbohydrate hapten, or the lipoprotein components, and those carbohydrate and lipoprotein antigens of normal cell surfaces, leading to a degree of tolerance because of a close antigen resemblance between hepatitis B surface antigen and 'self' antigens or alternatively initiating an autoimmune reaction.

Such an autoimmune reaction might be induced by hepatitis B infection because of a change in antigenicity of the hepatocyte cell membranes, due to alteration of existing antigens or the appearance of viral determinants. T lymphocytes responsive to such new antigen determinants could promote a B cell response to unaltered self antigens. The synthesis and release of the resulting autoantibody is subject in turn

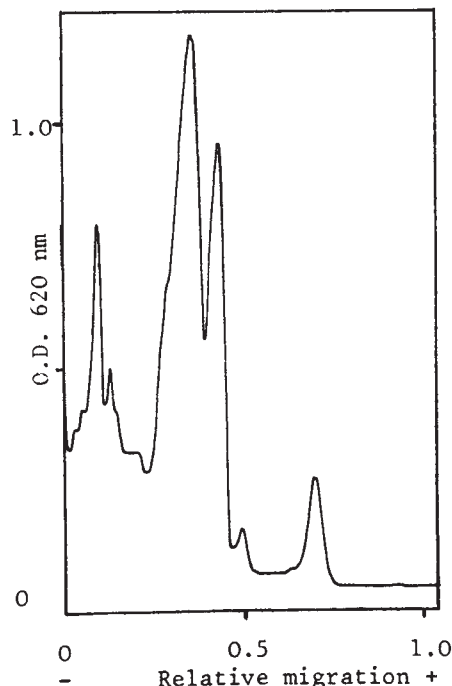
to control by suppressor T cells.

Liver-specific lipoprotein is a macrolipoprotein which is thought to be a normal constituent of the hepatocyte plasma membrane (Hopf *et al.*, *J. clin. exp. Immun.*, **16**, 117; 1974). Williams and colleagues at King's College Hospital and at the London School of Hygiene and Tropical Medicine found evidence of cell-mediated sensitisation to hepatitis B surface antigen in all patients with acute hepatitis B. Transitory sensitisation to liver-specific lipoprotein was detected in many of the patients.

These findings are consistent with the hypothesis that a cell-mediated response to hepatitis B antigen, present early on at the onset of acute hepatitis, is the cause of acute liver damage by a cytotoxic effect on virus-infected cells. If the response to liver-specific lipoprotein persisted, it could be responsible for progression to chronic liver damage. It is also postulated that the progressive liver damage of active chronic hepatitis is due to an autoimmune reaction directed against an hepatocyte surface lipoprotein which is initiated in most cases by infection with hepatitis B virus. This was investigated by Lee and associates (*Br. med. J.*, **1**, 705; 1975) who observed evidence of cell-mediated immunity to hepatitis B surface antigen in 62% of patients with antigen-negative active chronic hepatitis, suggesting a high frequency of previous infection with hepatitis B virus. A cellular response to the antigen was found in the majority of hepatitis B antigen-positive patients. Evidence of sensitisation to liver-specific lipoprotein was found in more than half of the patients, with a similar frequency in the two groups. These results are consistent with the hypothesis that infection with hepatitis B virus is important in initiating the disease in many cases of active chronic hepatitis and that sensitisation to the liver cell membrane antigen is responsible for the perpetuation of the liver injury. Thomson *et al.* (*Nature*, **252**, 721; 1974) demonstrated the killing of isolated rabbit hepatocytes *in vitro* when incubated with lymphocytes from 20 out of 22 patients with untreated active chronic hepatitis. Blocking experiments strongly suggest that the cytotoxicity is due to an immunological reaction directed at a cell surface antigen.

All these observations indicate that immunological mechanisms and the presence of antibodies reacting with various tissue components may well be involved in the pathogenesis of liver damage. It may therefore be undesirable to employ preparations of hepatitis B surface antigen which contain host cell components for immunisation against this infection.

Subunits of the antigen, in the form



Profile of the polypeptides of purified hepatitis B surface antigen after SDS-polyacrylamide gel electrophoresis. The separated polypeptides were stained with Coomassie Brilliant Blue.

of small polypeptides, on the other hand, offer an attractive proposition as immunogens. Such preparations would exclude genes of viral and cellular origin and could not be infectious. Several laboratories are investigating now the subunit structure of purified hepatitis B surface antigen by polyacrylamide gel electrophoresis and it has been shown that such subunits consist of polypeptides. It seems that the antigen contains between five and nine polypeptides ranging in molecular weight from approximately 15,000 to 120,000 (see figure). Work is currently in progress to determine if such preparations can be used as vaccines.

The primary sequence of the haptenic peptide of the surface antigen may also provide an approach for the development of a synthetic peptide, which when coupled to a macromolecular carrier could serve as a suitable immunogen. Once detailed data are available on the peptide and amino-acid composition of the antigen, it should be possible to define by animal immunisation the moiety responsible for the antigenic activity.

Immunisation against hepatitis B is now within reach but careful evaluation of the preparations in appropriate non-human primates is essential before small trials are undertaken in human subjects. These studies should be directed not only at the protective efficacy of such immunogens but they should include careful assessment of all their effects on the immune system. □

Actin and myosin in non-muscle cells

Secretion, motility and cell division

from Robert S. Adelstein

PROTEINS similar in molecular structure and biological properties to the muscle proteins actin and myosin have been identified in a large number of cells found in both vertebrates and invertebrates. Actin purified from non-muscle cells has the same monomeric molecular weight as skeletal muscle actin (42,000 daltons), contains a single residue of the unusual amino acid N^ε-methyl histidine and polymerises into filaments upon alteration of the ionic strength. The details of this polymerisation are not understood but it is thought that actin in non-muscle cells undergoes reversible polymerisation-depolymerisations unlike actin in muscle cells, which is thought to exist only as a filament.

Myosin from vertebrate non-muscle cells resembles muscle myosin (molecular weight 460,000) in being composed of heavy chains (200,000 daltons) and light chains (20,000 and 16,000 daltons in the case of fibroblasts, platelets and macrophages). Similar to muscle myosin, cytoplasmic myosin possesses an enzymic activity which catalyses the hydrolysis of ATP into ADP and inorganic phosphate. This ATPase activity is markedly enhanced at low ionic strength in the presence of Mg²⁺ by the addition of actin. It is noteworthy that cytoplasmic myosin interacts with muscle actin and *vice versa*.

Two major problems occupy the ever expanding number of workers attracted to this field: (1) the location of actin and myosin in non-muscle cells and (2) the function of these proteins (see also *Nature*, 253; 399; 1975).

Three important cell processes have been postulated to involve cytoplasmic actin and myosin: secretion, cell division, and cell motility. It is important to note that all the evidence presented to date is at best circumstantial, in that no direct evidence for the role of actin and myosin in any of these processes has been reported.

In a recent article Burridge and Phillips (*Nature*, 254, 526; 1975) presented evidence for the association of actin with the secretory granules of the adrenal medulla. These granules contain neurotransmitters which are released, following exocytosis into the blood stream. The authors succeeded in preparing membranes from partially lysed chromaffin granules which bound rabbit skeletal muscle actin (and very

small quantities of chicken smooth muscle myosin). Binding was indicated by finding the contractile proteins and lysed membranes in the same fraction following sedimentation in a sucrose gradient.

The authors supplemented these studies with electron micrographs showing lysed chromaffin granule membranes associated with rabbit skeletal muscle actin filaments (the original photographs being superior in quality to those reproduced in *Nature*). In muscle cells actin is uniquely attached to a Z-line so that 'decoration' of the actin filament with a fragment of myosin, heavy meromyosin, leads to the formation of polarised 'arrowheads' pointing away from the Z-line attachment site. Unfortunately this uniform polarity is lacking in the preparation of membranes and actin filaments examined by these authors raising the possibility that at least some of the filaments might not be uniquely attached to the membrane.

Puszkun and Kochwa in a preliminary communication (*J. biol. Chem.*, 249, 7711; 1974) presented data suggesting that contractile proteins are important in the release of neurotransmitters from brain tissue. They isolated a complex of actin and 'relaxing proteins' from rat brain synaptosomal membranes. The actin-'relaxing proteins' complex interacted with synaptic vesicles from brain, preloaded with ¹⁴C glutamate and presumably containing brain myosin. The results of the interaction (in the presence of Ca²⁺) were release of the labelled glutamate and activation of the Mg-ATPase activity of the myosin. The work is incomplete in that neither the relaxing proteins (presumably troponin) nor the myosin were positively identified. Moreover the release of ¹⁴C-glutamate is assumed to correlate with the secretion of neurotransmitter.

Evidence that actin in HeLa cells (human tumour cells grown in tissue culture) might play a part during one phase of cell division was first proffered by Schroeder (*Proc. natn. Acad. Sci. U.S.A.*, 70, 1688; 1973) who identified actin in the contractile ring of these cells. The identification was made by treating the cells with glycerine so they would be permeable to heavy meromyosin which interacted with actin filaments to form the unique arrowhead pattern alluded to above. Again, the evidence is circumstantial. The presence of actin implies, but does not prove, that it is functioning (perhaps with myosin?) to cleave the cells.

A role for actin in cell motility and phagocytosis was suggested by Boxer *et al.* (*New Engl. J. Med.*, 291, 1093;

1974) who investigated polymorphonuclear leukocytes isolated from a patient suffering multiple infections. These cells failed to migrate normally an ingested particles at a slower rate than controls. Actin isolated from these cells could not polymerise to the same extent as actin from the white cells of normal controls. It must be stressed that only a single patient's cells were examined and that the process of actin polymerisation using non-muscle actin is not well understood at present. But the principle of using a cell showing a defect in its motile behaviour as a source for studying actin is important.

Future experiments in this field should be directed at: (1) complete characterisation of the various proteins and their interaction; (2) the *in situ* identification of the contractile proteins using antibodies that have been shown to be specific for either actin or myosin by a number of rigorous criteria; (3) the removal of a specific link (for example, Ca²⁺ ATP, troponin) in the contractile mechanism which results in changes that can be related to cell function; (4) further studies looking for specific defects in the contractile proteins of abnormally functioning cells; (5) identification of a specific inhibitor of actin-myosin interaction which can be used *in vivo* (as well as *in vitro*) to study changes in cell function. The list, of course, can be ex-



A hundred years ago

Curious Phenomenon of Light

ROWING on Loch Lomond recently, above Luss, there were seen to the north-west, at an apparent distance of about 100 yards, two bright lines of prismatic light, 60° apart and on the level of the water. Their length seemed to equal the breadth of a rainbow. Their violet ends were towards each other, and were joined by a line of dull white light, to the middle of which the sun and the spectator were at right angles. Standing in the boat, the colour and brilliancy were lost, and only a diffuse white light was visible. The time was 10 A.M. The sun was hot, the sky cloudless, the air hazy and still, and the loch a mirror. This apparition fled before our approach for some minutes, till dispelled by a slight breeze, which rippled the water.

Luss WM. M'LAURIN
from *Nature*, 12, 26; May 13, 1875

panded but perhaps in the near future we shall have direct evidence as to the exact role of cytoplasmic actin and myosin.

Cell movement

from Robert Shields

SINCE the discovery that non-muscle cells contain actin it has become almost an article of faith among cell biologists that cell movements are somehow generated by actin-myosin interactions. Unfortunately non-muscle cells do not present their actin-myosin complexes in the nicely structured array seen in striated muscle and although microfilaments have been shown to contain actin-like proteins quite how this was translated into cell movement has been obscure until recently.

Cellular locomotion is most conveniently followed in tissue culture where it has been clear for some time that only limited areas of the underside of the cell approach closely to the surface of the culture vessel. Electron microscopy of a section of a cell's leading lamella reveals that electron dense plaques form on the inside of the cell membrane where the cell comes closest to and probably adheres to the culture vessel. These points can be seen in scanning electron micrographs just behind the ruffling cell membrane (Revel *et al.*, *Expl Cell Res.*, **84**, 207; 1974).

Soon after the formation of the plaques bundles of microfilaments can be detected running obliquely from the plaques towards the rear of the cell (Abercrombie *et al.*, *Expl Cell Res.*, **67**, 359; 1971). This arrangement of adhesion plaques near the cell borders and microfilament bundles running from the plaque parallel to the long axis of the cell has been beautifully confirmed in scanning electron microscope studies of the replicas of the undersides of untransformed BHK cells (Revel and Wolken, *Expl Cell Res.*, **78**, 1; 1973).

Because of the abundance of these points of adhesion near the advancing edges of cells it was natural to assume that cell locomotion might result from pulling of the microfilament bundles on the points of adhesion to the substratum. Evidence to support this idea has recently emerged in a series of papers from a group at Cold Spring Harbor. What these workers did was to raise antibodies to purified actin or purified myosin (which is no mean feat) and use indirect immunofluorescence to detect binding of actin or myosin antibodies to fixed cells by fluorescence microscopy. Untransformed 3T3 cells showed bright

staining with actin antibody along microfilament bundles which seemed to be close to the underside of the cell and orientated away from the cell periphery (Goldman *et al.*, *Expl Cell Res.*, **90**, 333; 1975). Similar patterns of staining were seen with anti-myosin antibody and in addition both antibodies stained the cytoplasm diffusely.

The intense patterns of staining along the microfilament bundles and the diffuse pattern over the body of the cell probably correspond to the two extreme arrangements of microfilaments encountered: bundles and a three-dimensional matrix that underlies much of the cell surface. The experiments strongly suggest that the bundles of microfilaments attached to the adhesion plaques contain both actin and myosin-like proteins and that the attachment of these bundles of filaments to the adhesion plaques could provide a mechanism for cellular locomotion.

Examination of cell replicas by scanning electron microscopy revealed another interesting feature. The underside of BHK cells transformed with polyoma virus showed few bundles of microfilaments or obvious points of cell substratum attachment, whereas such features were frequent in untrans-

formed BHK cells (Revel and Wolken, *ibid.*). Recent cell fractionation studies have also suggested that virally transformed cells have less actin associated with their cell membrane than do their normal counterparts (Wickus *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, **72**, 746; 1975). A series of indirect immunofluorescence experiments using actin antibody and rat embryo cells transformed with a newly isolated temperature sensitive mutant of SV40 virus showed what was happening. At the permissive temperature (where the cell exhibits its transformed phenotype) the brightly staining actin-containing bundles of microfilaments are almost completely absent but the diffuse pattern of cytoplasmic staining remains; however, after cultivation at the non-permissive temperature (where the normal cell phenotype is re-expressed) bundles of microfilaments, which stain with actin antibody, appear (Pollack *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, **72**, 994; 1975). From these results it would seem that transformed cells may have some defect in their ability to organise microfilament bundles and adhesion plaques and this may account for their altered cell-substratum adhesion and altered locomotion. □

Isotope clue to end of glaciation

by John Gribbin

THE use of measurements of oxygen isotope ratios to provide a clue to past temperatures is now a familiar tool of climatologists. But Kenneth and Shackleton have given a new twist to the technique in a study reported in *Science* (**188**, 147; 1975). They interpret a large change in the isotope pattern in planktonic foraminiferans from Gulf of Mexico sediments as arising from the influx of fresh water from the melting ice sheet at the end of the Wisconsin.

This certainly seems more plausible than trying to explain that particular feature in terms of a temperature change, since it would require an increase of 8 °C to 33 °C at a time (between 15,000 and 12,000 years ago) when, judging by other evidence, the Earth was not in a warm phase. The change in salinity caused by the influx of fresh water to the gulf would produce the same effect (if surface salinity fell by 10%), and the argument is strengthened by a comparison of shells of deep-water creatures with those that lived near the surface; the isotope ratios in the latter show five times the change found in the former.

The model also fits in well with present understanding of the geography of the ice sheet, which would have prevented a substantial easterly flow of meltwater until about 12,000 years ago, when the Hudson River system opened up; before that time, the chief escape route for meltwater would have been a southward flow through the Mississippi River system.

All this tells us little new about recent glaciation. The real interest, of course, is a possible technique for investigating the extent of earlier ice sheets. As Kennett and Shackleton summarise the work, "earlier phases almost certainly would have influenced salinities in the Gulf of Mexico and will provide information on the extent of earlier ice sheets, since it can be assumed that no substantial meltwaters would have entered the Gulf unless the southward extent of the ice sheet was greater than the latest Wisconsin limit about 11,200 years ago. Such an approach will be of particular value since little is known about earlier episodes of deglaciation, because succeeding glacial advances have redistributed the older deposits".

Carbon dioxide fluctuations

from Peter D. Moore

THE global movement of carbon between non-living and living matter is one of the many cyclic processes upon which man's industrial activities are having considerable effect. The combustion of fossil fuels accelerates one part of the 'natural' cycling of carbon; it replaces the respiratory activity of decomposer organisms, whose consumptive processes were impaired by anaerobic conditions during the accumulation of the organic detritus which is now coal, oil and gas. Industrial combustion, however, is almost certainly responsible for the observed rise in ambient atmospheric carbon dioxide levels over the past sixteen years in Hawaii. Baxter and Walton (*Proc. R. Soc.*, **A318**, 213; 1970) suggest an increase of 22% on pre-industrial levels of atmospheric CO₂. Evidently the increased output of carbon dioxide, resulting from increasing industrialisation, is not being fully compensated by CO₂ consumption in other global reservoirs.

The major potential carbon reservoirs which might be expected to increase consumption of atmospheric CO₂ as concentrations rise are the oceans and the living organic matter of the planet. It is obviously a matter of considerable urgency to develop predictive models of this equilibrating system in order to determine the likely outcome of man's industrial activity; several such models now exist (for example, Fairhall, *Nature*, **245**, 20; 1973; Broecker *et al.* in *Impingement of Man on the Oceans*, edit. by D. W. Hood; Wiley, 1971). Opinions differ as to the likely influence of the additional carbon load upon the chemistry of ocean water (see Whitfield, *Nature*, **247**, 523; 1974).

World biomass variations are not easily estimated. One might predict increases in photosynthetic production as a consequence of enhanced CO₂ levels, but one must set against this the possibilities of reduced productivity because of raised SO₂ and O₃ concentrations in the atmosphere of industrial countries and also the destruction by man of many naturally productive areas. The production: respiration ratio of the world's biota could be moving in either direction. Reinert (and several other contributors to *Brookhaven Symp. Biol.*, **25**; 1973) have drawn attention to the general lack of reliable data and the wide disparity among estimates of such carbon reservoirs as living biomass and detritus pools, let alone transfer rates. Attempts have also been made to use tree growth rings as indices of forest production (for example, Whittaker *et*

al., *Ecol. Monogr.*, **44**, 233; 1974) and Farmer and Baxter (*Nature*, **247**, 273; 1974) have used the isotope ratio of ¹⁴C/¹²C in efforts to identify the contribution of fossil carbon to the growth rings of trees in Britain.

In this issue of *Nature* (page 136) Hall, Ekdahl and Wartenburg use a method which has been applied in many smaller scale studies to estimate trends in the biotic metabolism of carbon in the Northern Hemisphere. Their idea is based upon the flux study concept as used for the determination of forest respiration by Woodwell and Dykeman (*Science*, **154**, 1031; 1966), but rather than diurnal changes, they use seasonal fluctuation in ambient CO₂ levels (values are corrected by subtraction of the estimated residuum of industrially derived CO₂ after partition with the oceans). Increasing CO₂ levels in autumn and winter provide a measure of the respiration of the hemispheric ecosystem and decreasing levels in spring and summer give a measure of net photosynthesis. Using the data from the Mauna Loa observatory, Hawaii, they calculate hemispheric net production and respiration for the sixteen years over which data are available and they find no significant change during this period. This suggests that either the total biomass of the hemisphere is unchanged, or any loss of productivity due to pollution or changing land use has been compensated by an increase in the productivity of the remainder. It is possible to argue about the best figures to use for industrial carbon output and the equilibration of this carbon with the oceans (the prime considerations in the adjustment of the Mauna Loa data) but the authors claim that the outcome of their calculations is relatively insensitive to possible errors in these data. Their plea for a more extensive worldwide monitoring of atmospheric CO₂ fluctuations as an aid, among other things, to the detection of any major variation in global carbon metabolism, should be supported by anyone with a sense of responsibility for the environment.

Statistics and climatic change

from Michael Kelly

GREAT advances are currently being made in the study of climatic change. This progress is attributable not only to greater academic and, in certain countries, governmental interest in the subject but also to the increasing use of powerful statistical tools in the analysis of climatic time series.

Post-glacial climatic change can be viewed as movement between atmospheric circulation states of predominantly strong, westerly airflow (leading

to warming of higher latitudes in the sector or sectors affected, as occurred globally in the first half of the twentieth century) and those of predominantly weaker, more meridional airflow (leading to cooling). Characteristic time scales or periodicities can often be attached to variations in climate; this had led to many attempts to explain the observed persistence of a particular circulation state for periods of months to centuries and also the change to a different state at fairly regular intervals. Climatic cycles may eventually prove of great value in climatic forecasting but until a physical reason for their continued existence is established, such forecasts can only be tentative.

The search for the causes of climatic change and climatic cycles is proceeding on two fronts: computer modelling of the atmospheric circulation and statistical analysis of climatic data. The potential of both of these approaches is great but, at present, most computer models are too coarse to differentiate between different circulation states experienced during the last millennia and it is the second, more traditional, approach to the study of climatic change that has been greatly improved by the availability of new statistical methods and that is rapidly increasing understanding of climatic change.

A recent paper by Trenberth (*Q. Jl R. met. Soc.*, **101**, 55; 1975) illustrates the use of sophisticated data analysis in a study of ocean-atmosphere interaction in the region of New Zealand and Australia. Empirical orthogonal function analysis was used to identify the major sea level pressure (SLP) anomaly patterns, that is, atmospheric circulation states, and the major sea surface temperature (SST) anomaly patterns for the period 1959-1972. Spectral and cross-spectral analysis of time series of the pattern coefficients then revealed relationships, both current and lag, between the SLP and SST anomaly patterns, associated with a quasi-biennial periodicity which has been observed in many climatic parameters. These relationships enabled Trenberth to construct a sequence of SLP and SST anomaly charts for the two-year cycle, which showed that the SST anomalies were largely caused by oceanic advection resulting from the wind drag associated with the different SLP patterns.

This research did not indicate that feedback from ocean to atmosphere was of great importance in the region studied. But work in other areas, particularly the Pacific and North Atlantic, has shown that anomalous sea temperature patterns often reinforce the atmospheric circulation states that produce them. Although this positive feedback can explain the persistence of certain atmospheric circulation states,

it cannot explain the observed regularity of many climatic cycles. If this is to be explained in terms of ocean-atmosphere interaction, some form of complex negative feedback between ocean and atmosphere must be postulated.

Complete nuclear isobaric quintet

from P. E. Hodgson

If nuclei of the same mass (isobars) are grouped together, it is found that their states can be conveniently classified using the isobaric spin vector T , analogous mathematically to ordinary spin. States of the same structure in different isobars have identical nuclear quantum numbers, including isobaric spin, and differ only in the third component T_3 of the isobaric spin vector. This quantum number T_3 is directly related to the excess of neutrons over protons, thus $T_3 = \frac{1}{2}(N - Z)$.

For example, the ground states of ^{23}Mg (12 protons and 13 neutrons) and ^{23}Al (13 protons and 12 neutrons) have the same nuclear structure and $T = \frac{1}{2}$, but the former has $T_3 = \frac{1}{2}$ and the latter $T_3 = -\frac{1}{2}$. These states constitute an isobaric spin doublet. Some of the higher states of these nuclei have $T = 3/2$, and then as before $T_3 = \frac{1}{2}$ and $T_3 = -\frac{1}{2}$ correspond to states in ^{23}Mg and ^{23}Al of the same structure, and in addition there are states of the same structure to be found in ^{23}Na (with $T_3 = 3/2$) and ^{23}Si (with $T_3 = -3/2$). These four states comprise an isobaric spin quartet. The states of nuclei with an even number of nucleons can be similarly classified into isobaric singlets, triplets, quintets and so on.

Analysis of the structure of these isobaric spin multiplets, as they are called, showed that for states of pure isobaric spin the mass excesses of nuclei in a particular multiplet are given by the simple quadratic expression

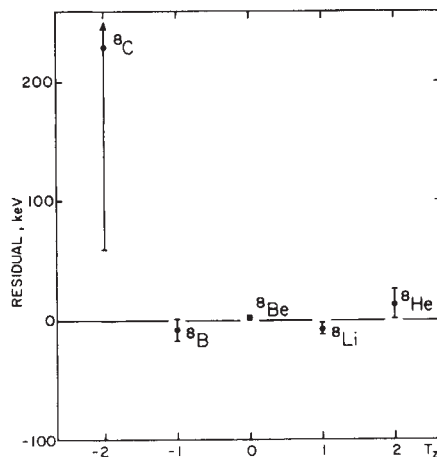
$$\Delta M = a + bT_3 + cT_3^2$$

where a , b and c are constants. Multiplets with up to three members automatically satisfy this relation, which is therefore not useful, but higher multiplets provide a means of testing its accuracy. Thus if the masses of all four members of a quartet are known then any three of them determine a , b and c , and from these the mass of the fourth can be calculated and compared with the measured values.

Over the past few years several such quartets have been measured, and on the whole have confirmed the above formula to a high degree of precision with some small systematic discrepancies that can be explained by higher-order effects.

A more severe test of the formula

is provided by higher multiplets, but this is difficult experimentally because the members of these multiplets with higher T_3 are usually states of highly unstable nuclei. Recently however a complete set of masses for the quintet in nuclei of mass 8 comprising the lowest $T = 2$ states in ^8C , ^8B , ^8Be , ^8Li and ^8He has been obtained by Robertson and Chien at Michigan State and Goosman at Brookhaven (*Phys. Rev. Lett.*, **34**, 33; 1975) and these have been used to test the isobaric mass formula. If the deviations from the above formula are represented by extra terms dT_3^3 , eT_3^4 , and so on, then a quartet determines one of these terms and a quintet two.



Amounts by which individual masses exceed those given by a weighted fit of the quadratic isobaric mass equation to the masses of the $A = 8$ quintet.

The five masses were first fitted by the above equation with the addition of the dT_3^3 and eT_3^4 terms, and it was found that $d = -18 \pm 14$ keV, and $e = 13 \pm 7$ keV, indicating that e , but not d , is probably significantly different from zero. The residuals in a three parameter fit to the above formula are plotted in the figure, and the odd-even staggering corresponding to a non-zero value of e is apparent. It is clear that a more accurate value of the mass of ^8C is needed, and this would increase the sharpness of the test.

There are several effects that could cause deviations from the simple three-parameter equation. One of these, the isospin mixing of $T = 0$ and $T = 2$ states in ^8Be , is readily calculable, and gives $d = 0$ and $e \approx -1$ keV. This is consistent with the result of a fit to the measured masses when d is set equal to zero. Remaining small differences may be caused by increasing Coulomb repulsion in the neutron-deficient members of the quintet.

The results from the first complete quintet thus confirm the high accuracy of the simple isobaric mass formula, and indicate that the very small deviations can be accounted for by calculable higher order effects.

Does San Miguel threaten San Diego?

from Peter J. Smith

THE San Miguel Fault in Baja California (Mexico) strikes roughly parallel to the other major faults in the San Andreas system and, like those other faults, presumably results from the north-westerly movement of the Pacific Plate with respect to the North American Plate. The detailed relationships between the San Miguel Fault and faults further to the north in the United States have not yet been established precisely, however, although Wiegand (*Bull. Ass. engng Geol.*, **7**, 107; 1970) and others have proposed that a shear zone which begins with the San Miguel Fault in the south-east extends right through to the Inglewood fault zone in the north (the site of the Long Beach earthquake of 1933). This suggested shear zone would also include the Rose Canyon fault zone which passes through San Diego and Tijuana.

If these connections are proved, it follows that the behaviour of the San Miguel fault zone is pertinent to the problem of seismic hazard in San Diego; and for this reason Reyes *et al.* (*Geophys. Res. Lett.*, **2**, 56; 1975) have, since 1970, been measuring the micro-earthquake activity at various sites in the vicinity of the fault. They found that the overall rate of microearthquake activity at the south-eastern end was about 100 events a day (December 1970–December 1973), although some individual rates rose to 600 and many were significantly higher than those observed along most of the San Andreas Fault in southern California. By contrast, the overall activity towards the more critical (for San Diego) north-western end of the San Miguel Fault was about 20 events a day, with individual rates of up to 70 a day.

The San Miguel fault zone is thus microseismically active. But what does it mean in terms of larger, potentially destructive earthquakes? The problem, as Reyes and his colleagues acknowledge, is that microearthquake activity is not necessarily indicative of long term seismic activity. For example, the 1857 Californian earthquake occurred along a section of the San Andreas Fault which is now 'locked' and exhibits very little microseismicity, although in other sections of the fault microearthquake rates are consistent with the seismicity observed over the past few decades. But although precise correlations cannot be made, the activity observed by Reyes *et al.* suggests that the seismic risk to San Diego and Tijuana from the north-western San Miguel fault zone should not be taken too lightly. □

Organ culture

from Michael Balls and
Marjorie A. Monnickendam

The British Society for Cell Biology held a symposium on organ culture in biomedical research at the University of East Anglia on 8–11 April, 1975.

In the opening session, Dame Honor Fell (University of Cambridge) outlined the historical development of organ culture, and stressed that, though some well-meaning organisations were currently advocating the use of organ and cell cultures to replace experiments on live animals, there were insuperable objections to this as a general policy. Although organ culture experiments gave useful information which could not be obtained from animal experiments, there were many factors operating *in vivo* which could not be satisfactorily mimicked *in vitro*.

Giselle Hodges (Imperial Cancer Research Fund, London) reviewed the range of organ culture techniques currently in use, and J. Simnett (University of Newcastle-upon-Tyne) stressed the importance of care in the handling and preparation of tissues while setting up cultures.

The usefulness of organ culture in morphogenetic studies was shown in papers on germinal tissues (R. Dubois, Institut d'Embryologie, Nogent-sur-Marne), the kidney (Y. Croisille, Nogent-sur-Marne), cartilage and bone (Sylvia Fitton Jackson, Strangeways Laboratory, Cambridge), nervous tissue (S. Aparicio, University of Leeds), and skin and its derivatives (P. Sengel, University of Grenoble; A. Melcher, University of Toronto). C. Cruickshank (MRC Skin Unit, Birmingham) reviewed tissue culture studies on cell differentiation and function in adult skin, and W. de Voogd van der Straaten (Rijks Universiteit, Utrecht) discussed the suitability for cell kinetics studies of the exfoliated chick tracheal epithelium *in vitro*.

A number of contributions demonstrated the value of organ culture techniques in endocrinology, and the problems which can arise. Isabel Forsyth and E. Jones (National Institute for Research in Dairying, Reading), and R. Dils (University of Nottingham) discussed the hormonal induction of milk fat synthesis and changes in enzyme activities and nucleic acid synthesis in cultured mammary glands. Margaret Ryle (Birmingham and Midlands Hospital for Women) outlined work on endocrine aspects of the ovary, comparing the results of short-term incubation and long-term culture, and drawing attention to the problems which arise because of

the need to support the tissue and the use of sera in media. *In vitro* investigations on the developing and adult pars intermedia were described by P. Chatterjee (St Mary's Hospital Medical School, London), and Ilse Lasnitzki (Strangeways Laboratory) and D. Cannon (St Helier Hospital, Carshalton) discussed hormone effects and hormone metabolism in the prostate gland. The pig costal method for quantifying the activity of serum somatomedin (the active intermediary of growth hormone) was described by J. Williams (Institute of Child Health, London). Studies on the foetal rat pancreas concerned the control of insulin synthesis and release by islet beta cells in organ culture and in monolayer cell culture (A. Lambert, University of Louvain) and amylase production by acinar tissue maintained in chemically defined medium in circumfusion organ culture (L. Murrell, University of Tennessee).

J. Reynolds (Strangeways Laboratory) opened a more clinically oriented session with a discussion of *in vitro* methods for studying the role of hormones and vitamins in bone resorption. G. Easty (Institute for Cancer Research, London) then discussed the production of osteolytic substances by tumours. In a fascinating talk, he related *in vitro* and *in vivo* observations, showing that the release of osteolytic activity (due to prostaglandins and a high molecular weight component) by human breast tumours *in vitro* was inhibited by high doses of aspirin, and that, in an animal tumour model, bone lysis and hypercalcaemia (though not tumour growth) were inhibited by aspirin and related drugs. Etienne Wolff (Collège de France, Nogent-sur-Marne) described the methods he pioneered for the long-term maintenance of human tumours *in vitro*, and reviewed the results of investigations on tumour growth, growth inhibition, and the effects of ionising radiations. Dorothy Easty (Institute for Cancer Research) tackled the problem of quantifying tumour invasiveness by examining the ability of suspensions of tumour cells to penetrate various tubular and membranous tissues, and measuring the effects of various drugs on invasiveness. Elizabeth Defries (Imperial Cancer Research Fund) reviewed chemical carcinogenesis in organ culture, where the major problem is to determine the extent to which morphological changes *in vitro* are related to the ability of the tissues to form tumours *in vivo*. J. Masters (The Royal Infirmary, Edinburgh) critically reviewed an *in vitro* method for predicting the hormone sensitivity of human breast tumours. His cautious approach was backed up by J. Dickson (University of Newcastle-upon-Tyne),

who pointed out that, although *in vitro* biochemical responses of human tumours to drugs and/or hyperthermia indicate the existence of sensitive populations of cells within tumours, because of the complexities of the host–tumour relationship, one could not assume that treatment effective on a tumour *in vitro* would be effective *in vivo*.

The effects of external agents on cultured tissues were described by several speakers. Sylvia Reed (MRC Common Cold Unit, Salisbury) discussed the growth of viruses and mycoplasmas in organ culture. The technique has proved helpful in a number of ways; for isolating and growing viruses which would not grow in permanent cell lines, for examining the role of local immunity by comparing virus growth in tissues from immune and non-immune animals, and for examining interactions between viruses and mycoplasmas in mixed infections. There was no correlation between the virulence of viruses *in vivo* and their cytopathic effects *in vitro*. Lynne Reid (Cardiothoracic Institute, London) discussed the synthesis and secretion of glycoproteins by human bronchial submucosal glands, comparing normal and hypertrophied glands, and considering the effects of various drugs on them. T. Rae (Strangeways Laboratory) described a method for using rat foetal knee joints in culture as a means of measuring the tolerance of tissues towards materials used for orthopaedic implants. D. Lucas (University of Manchester) reviewed work on the effects of X-irradiation on embryonic, normal adult, and neoplastic tissues.

Although most of the discussion in the symposium was concerned with mammalian and human tissues, the host institution contributed three papers which illustrated the potential value of long-term organ cultures of amphibian tissues. These were concerned with the control of renin production and release (R. Worley), activation and blockade of hepatic adrenoceptors (D. Brown), and the hepatotoxic effects of high concentrations of paracetamol (S. Gater).

Concluding the proceedings, L. Franks (Imperial Cancer Research Fund) said that this should be the last symposium concerned with organ culture methods, for, like electron microscopy, the technique had now come of age: organ culture should now be regarded as a standard method which could be applied to most biological problems. He concluded that this was largely due to Dame Honor's unfailing ability to give good advice, and to her success in passing on her belief in the importance of a critical appraisal of an apparently ordinary object looked at from an extraordinary angle.

review article

Sex control in silkworms

Vladimir Strunnikov*

The production of hybrid silkworms yielding a very high proportion of male larvae is of great importance to the silk industry, because of the very much higher yield of silk from males. Strains giving as many as 98% male offspring have now been developed from mutants with translocations of the X chromosome.

THE problem of sex control in the silkworm *Bombyx* is one of great practical and theoretical importance. Male silkworms are more viable and produce more silk than do females. The commercial breeding of exclusively male silkworms would mean producing 15–18% more silk than is now obtained from bisexual strains. Furthermore, the use of sex control methods would solve the technically complex problem of preparing interbreed hybrids, since there would be no need for the present labour-intensive and inaccurate methods of separation of females and males.

Female silkworms are heterogametic (XY) and the males homogametic (XX). (The X and Y chromosomes of lepidoptera are often known as Z and W respectively.)

A variety of environmental agents, irradiation and thermal shock for example, disturb the normal processes of germ cell maturation and fertilisation and form the basis for the experimental control of sex in silkworms. In this article I will review the application of 'chromosomal engineering' for the production of unisexual and 'autosexing' strains of *Bombyx mori*. Four techniques will be considered, two of which are already of commercial interest.

Artificial parthenogenesis

Artificial parthenogenesis was discovered in silkworms by Tikhomirov in 1886. In his experiments, as in those of many other investigators, parthenogenetic eggs developed abnormally, and only in rare cases hatched into larvae.

In 1940 Astaurov¹ developed a simple and very reliable method for obtaining parthenogenetic offspring. He dissected eggs from the ovarioles and, after ageing for 10–12 h, immersed them in a water bath at 46 °C for 18 min. After this treatment the eggs were kept in water at room temperature for 5–10 min. The activated eggs were then kept at 15–17 °C and 80% r.h. for 3 d, and then under normal conditions.

When laid the silkworm egg's nucleus is blocked at metaphase I. Normally fertilisation stimulates both the completion of the reductional first meiotic division and the subsequent equational second division. Heat treatment of unfertilised eggs apparently stimulates these maturation divisions and at the same time destroys the first division spindle.

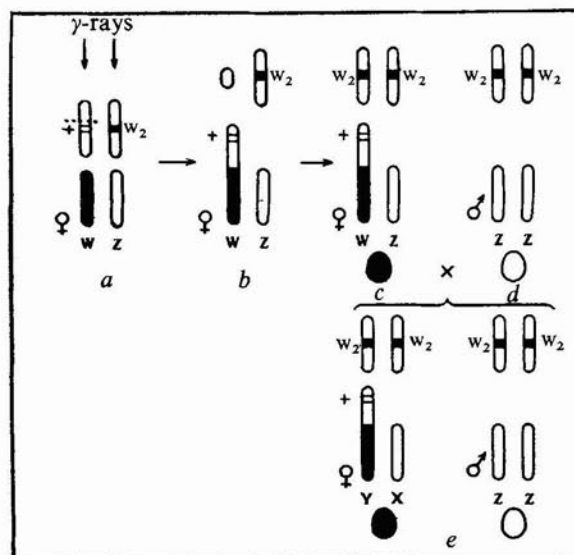
The second meiotic division is normal and diploid pronuclei are formed. These pronuclei are all XY and hence all parthenogenetic individuals are female and identical in genotype to their

mother. By selection Astaurov² has produced strains from which 95% of eggs will develop, after activation, by ameiotic parthenogenesis. About 98% of the larvae develop into adult moths—a survival rate better than that of most bisexual hybrid strains.

Spontaneous parthenogenesis in silkworms ending in hatching is very rare^{3,4}. The mechanism of spontaneous parthenogenesis differs from the previously described induced ameiotic parthenogenesis since both meiotic divisions occur normally—diploidy being restored by the fusion of daughter cleavage nuclei. Thus spontaneous parthenogenesis is meiotic. Its frequency can be increased by various agents, such as low temperature (–5 to –17 °C), high temperature (44–50 °C) or exposure to CO₂^{5,6}. These treatments also induce meiotic parthenogenesis in 95% of eggs, which reach the stage of the pigmentation of the serosa, and only 2–3% of eggs are induced towards ameiotic parthenogenesis. With an increase of exposure doses the eggs are stimulated only towards ameiotic parthenogenesis.

After meiotic parthenogenesis two types of embryo are formed; XX and YY, both being completely homozygous. YY embryos are inviable. Presumably because of homozygosity

Fig. 1 The production and use of Y:10 translocation with w_2^+ for the production of a strain in which all male eggs are white and all female eggs coloured.



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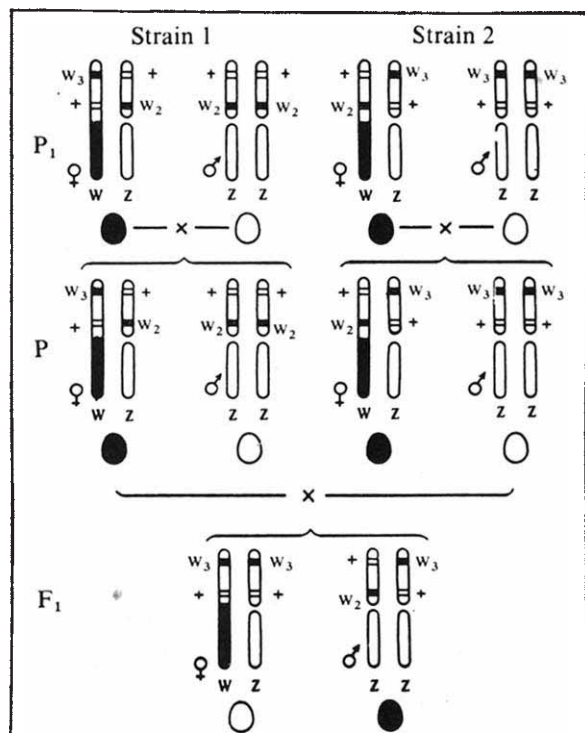


Fig. 2 Construction of two sex-marked strains using the Y:10 translocation and the linked egg colour mutants w_2 and w_3 . Both strain 1 and strain 2 breed true and have coloured female and white male eggs. Their F₁ has coloured male and white female eggs.

for lethal or semilethal mutations only 8–9% of XX embryos hatch; they are, of course, all males. Because of the greater viability of these heterozygous females, compared with the homozygous males, the larval sex ratios are usually of the order of 93 males to 7 females.

Thus, depending on the type of activation technique used silkworms which are either entirely females (and heterozygous) or predominantly males (and homozygous) can be produced at will.

Dispermic androgenesis

Dispermic androgenesis in silkworms was discovered independently in Japan by Hasimoto⁷ and in the Soviet Union by Astaurov⁸. As a result of heat treatment of eggs one hour after laying (for 1 h at 40 °C)⁶ or of X-irradiation of oocytes and heat treatment after insemination (18 min at 46 °C)⁸ the maturation divisions are so disturbed that no female pronuclei are formed. Since silkworms are polyspermic the opportunity then arises for two different male pronuclei to fuse and form a diploid zygote nucleus. Since the male silkworm is homogametic all individuals developing by androgenesis are male.

The irradiation of oocytes in the body of a female moth before copulation with the doses of 80–90 krad, after further heating, yielded twice or three times as many androgenous caterpillars as were obtained from non-irradiated but similarly heated eggs. The embryos formed after the fusion of an irradiated female pronucleus with a male pronucleus are unviable. That is why the irradiated oocytes give only androgenous male offsprings^{9,10}. The timing of the subsequent heat treatment is critical—it should coincide precisely with prophase of the second division (70 min after laying at 23 °C). Under optimal experimental conditions as many as 20–30% of treated eggs may develop as androgenous males¹¹.

Double insemination of females by different males allows normally viable androgenous offspring to be produced following fusion of sperm pronuclei from different fathers¹².

Neither parthenogenesis nor dispermic androgenesis is yet a

practical technique for producing a single sex in silkworms on a commercial scale. Two techniques that serve the same end and are commercially viable will now be described.

Development of sex-marked breeds

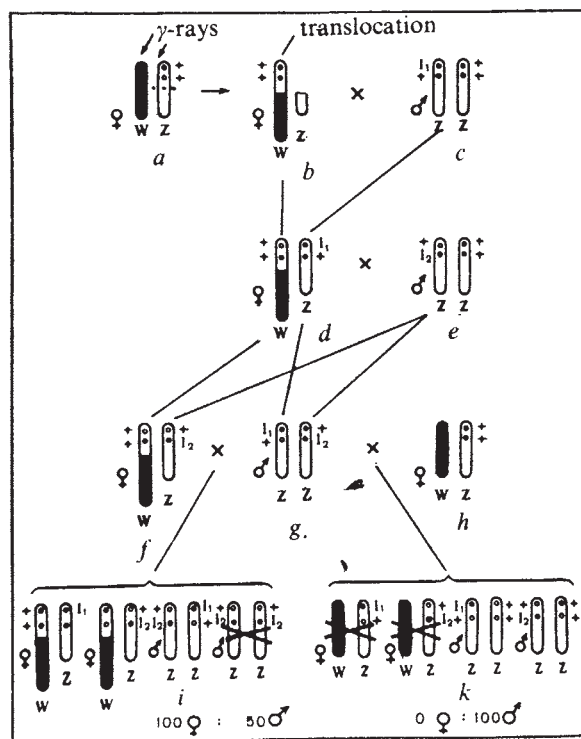
One of the first steps toward evolving methods of sex control on a commercial scale was the development of sex-marked silkworm breeds. At first one such breed, sex-marked in the egg stage, was obtained by Tazima¹³ and later two breeds of this kind were evolved in the Soviet Union¹⁴. Although these breeds have never had a wide distribution in silkworm breeding, the methods used in making them will be described below to make it easier to understand the need for, and the essence of, more complicated methods for obtaining faultless sex-marked breeds.

On chromosome 10 of the silkworm there is a gene, w_2 , the wild type allele of which determines the formation within the developing embryo of a dark pigment giving the embryos their normal coloration. A recessive mutation at this locus blocks the formation of this serosal pigment and the eggs remain white.

An irradiation induced translocation has transferred the w_2 + allele onto a Y chromosome (Fig. 1). Strains in which both the chromosomes 10 are structurally normal and the Y chromosome carries the translocation are viable, although somewhat reduced in weight, despite aneuploidy for the region of chromosome 10 distal to the translocation break point. If the chromosomes 10 are both marked with the recessive w_2 the translocation females will produce, on crossing to homozygous w_2 males, two types of egg—white and coloured—which will develop exclusively into males and females respectively.

Although a practical method for distinguishing male and female embryos, this technique suffers from the fact that the w_2 homozygous males have lowered viability. For this reason 'autosexing' strains have been developed which have dark female and white male eggs but which, when crossed, give dark male eggs and white female eggs¹⁵.

Fig. 3 The construction and use of a balanced X-linked lethal stock by the use of an X:Y translocation which covers both lethal loci. The stock is balanced (bottom left) with a 2:1 sex ratio. On outcrossing only male progeny are viable (bottom right). See text.



Aneuploid segregants from the Y:10 translocation that carry the translocated Y:10 element and a single normal chromosome 10 are viable and fertile females. In strain 1 the translocated chromosome is marked with w_2^+ and w_3 . w_3 is a recessive mutation also localised at chromosome 10 which also results in white eggs. It fully complements with w_2 . The normal chromosome 10 of strain 1 females is marked with w_2 and w_3^+ . The males are homozygous for w_2 . Strain 2 carries the same translocation but now marked with w_2 and w_3^+ and with its normal chromosomes 10 marked with w_2^+ and w_3 . Both strains breed true and have coloured female eggs and white male eggs. Yet when strain 1 females are crossed to strain 2 males all male eggs are wild type and all female eggs are white (Fig. 2).

The advantage of these strains over that previously described can be seen from a consideration of the logistics of silkworm breeding. During the production of pure breeds the females are more important than the males—in these strains the females have coloured eggs and full viability. But for silk production males are more important than females and, in the strain hybrid, it is they that have the wild type egg colour. The translocated females resume their normal weight with the restoration of the balance of the tenth autosomes.

Automatic photoelectric devices can sort out hybrid eggs according to colour, and hence sex, at a rate of 140 eggs⁻¹. This may seem high yet it is inadequate for commercial breeding which requires the separation of tens of billions of eggs. This method is only used for the preparation of hybrid stock, hybrid males for commercial breeding being isolated by a different genetic method.

Use of balanced sex-linked lethals

X-linked lethals have been used in two ways to produce offspring that are entirely male. Males heterozygous for an X-linked lethal give 2:1 male:female sex ratios when crossed to females. Males heterozygous for two different sex-linked lethals, will give no viable female progeny. The construction of such stocks is normally impossible but can be done, in silkworms, by either

dispermic androgenesis or by the use of duplications of some regions of X-chromosomes.

I have used¹⁶ dispermic androgenesis to construct males doubly heterozygous for X linked lethals. These males, when crossed to females, gave a sex ratio of 98.6 males to 1.4 females.

A more efficient method of producing males heterozygous for two different lethals on their X chromosomes has been developed using a translocation of the left end of the X chromosome on the Y chromosome. The translocated portion of the X carries the wild type alleles of both lethals and translocation bearing females are fertile despite the sex chromosome aneuploidy. The genetic programme for making the balanced lethal stock is shown in Fig. 3. Females of the balanced stock all carry the X:Y translocation and a normal X chromosome marked with either lethal 1 or lethal 2. The males are all heterozygous for both lethal 1 and lethal 2. This stock breeds true but when the males are outcrossed to wild type females nearly all female zygotes die as a result of the expression of either one of the lethals. The 0.1% or so females that survive do so as a consequence of recombination between the lethal loci.

The use, for silk production, of hybrid strains which produce entirely males will greatly benefit silk yield. Both the Y:10 and X:Y translocations described have been introduced into commercial breeds of the silkworm and are being used in commercial breeding in the Soviet Union.

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articles

Interaction between the daily heat balance and the tidal cycle

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In a sea area consisting partly of periodically drying tidal flats, the semidiurnal lunar tide, interacting with the daily variation of solar radiation, gives rise to both a beat in the amplitude of the daily water temperature cycle with a period of 14.76 d, and a variation of the daily mean temperature, with the same period. This is shown theoretically and by observation.

In general, the frequency spectrum of temperature fluctuations at the Earth's surface is dominated by annual and daily frequencies and their higher harmonics. These peak frequencies of the spectrum originate from, and can be computed from, the variation of solar radiation at a given latitude¹. The rest of the spectrum can be attributed to 'atmospheric noise' (such as varying cloudiness and advection of different air masses, for instance by depressions, interchanging heat with the Earth's surface).

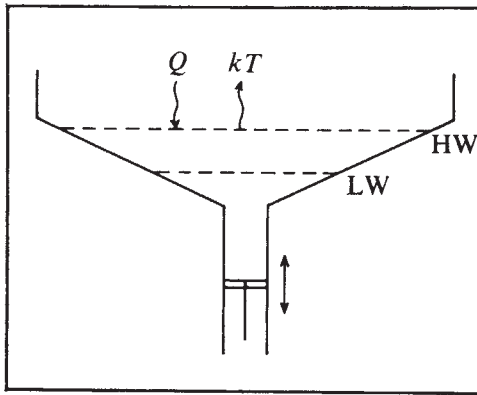


Fig. 1 Sketch of the model experiment described. HW, high water; LW, low water.

With respect to the sea surface the advection by periodic currents, such as those produced by the tides, may, in a non-homogeneous temperature field, also produce a peak in the frequency spectrum; the frequency being the same as that of the current involved if the temperature is measured in a frame of reference fixed to the sea floor. The latter fluctuations, however, disappear if the temperature is integrated over an area large as compared with the scale of the periodical currents.

This shows that the latter fluctuations arise from an internal redistribution of water parcels but not from an external forcing agent such as the absorption of solar radiation and heat exchange with the atmosphere. It is, however, possible that in certain areas periodic (tidal) currents produce externally forced temperature fluctuations with frequencies that are sums or differences of the tidal and daily frequencies and their higher harmonics. This will be the case in those areas along the coast, where the effect of the tide is to produce a periodical flooding of tidal flats. This causes a periodical change in the surface area through which solar radiation penetrates and heat is exchanged with the atmosphere.

Knowledge of the heat balance and temperature cycle of such a tidal-flat sea is relevant to the biology of these areas; temperature is one of the most important physical factors in its ecosystem. Moreover, in planning power stations which will discharge heated effluents in areas of tidal flats, the special character of the heat balance should be taken into account.

Theoretical considerations

In a sea which covers tidal flats the tidal currents cause both a periodical translation of water masses and a periodical flooding of the tidal flats. In consequence, the area of interface between the sea water and the atmosphere will differ during different phases of the tide. At low tide a given volume of water will collect in deeper tidal channels and will thus have a smaller surface area than at high tide when part of the same volume will be spread out over the tidal flats.

To study the influence of such a periodically varying surface area on the heat balance of the water volume, we have devised a simplified model experiment which is assumed to represent the real situation in a tidal-flat sea.

The floor of a reservoir containing a volume V , of water of density ρ and specific heat c , slopes gently from the sides towards a hole in the middle (Fig. 1). The surface area of this volume of water can be varied by working a piston contained in a tube under the hole, up and down with a frequency, ω_2 . The amplitude of the piston displacement is such that the water surface never reaches the sides of the reservoir and never becomes as small as the cross section of the hole. It is supposed that there is a varying absorption of (solar) radiation and a varying heat exchange between the water volume and the overlying air. Furthermore, it is assumed that the volume is well

mixed in all directions so that it has at any moment a uniform temperature, T . The heat flux density, Q , through the water surface is then approximately given² by:

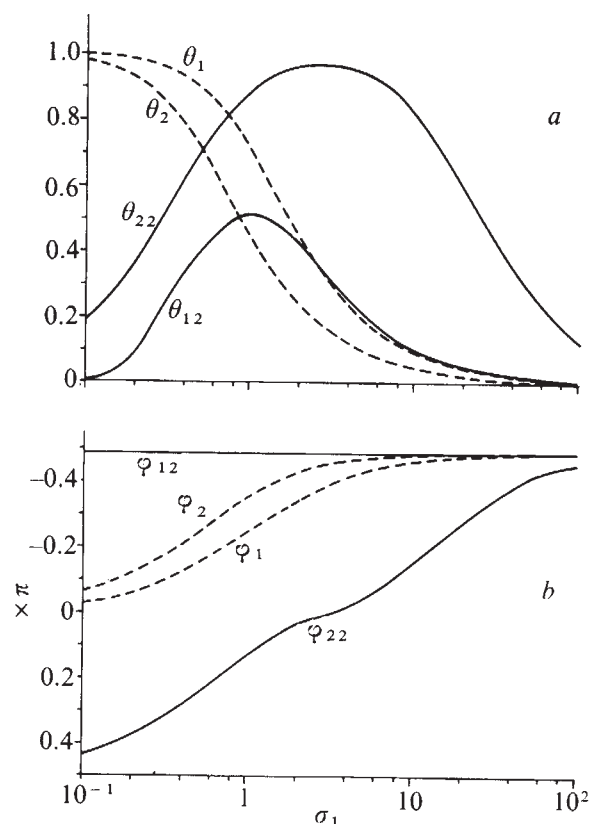
$$Q(t) = \sum_{n=-\infty}^{+\infty} q_n \exp(in\omega_1 t) - kT(t), \quad q_n = q_n^* \quad (1)$$

where q_n is a (complex) amplitude of the Fourier series in $\omega_1 t$ (ω_1 is the daily frequency). The factor k is a bulk parameter^{2,3} representing all the processes by which heat is exchanged with the atmosphere, such as long wave radiation, (turbulent) conduction, and evaporation. In general, k will be time dependent, but here it is assumed to be a constant. For $n \neq 0$ q_n contains both the daily variations in solar radiation and the effects of daily fluctuations in air temperature, humidity and wind velocity. We will, however, assume that these latter effects are of minor importance so that $q_n (n \neq 0)$ is only determined by the daily variation in solar radiation. Then, for a cloudless sky, q_n can be obtained easily for a given latitude and time¹. For $t = 0$ at midnight, all q_n are real whereas for odd absolute values of n greater than 1 they are zero. For $n = 0$, q_0 represents the constant part of the heat income. It is, however, only approximately a constant because it varies during the course of the year and, on a smaller time scale, because of varying atmospheric conditions. It follows from equation (1) that the equilibrium daily mean temperature of the water mass is given by q_0/k (refs 2 and 3). We will assume that T represents the deviation of the water temperature from this equilibrium value.

Now, the rate of change in temperature of a volume, V , is given by:

$$\frac{dT}{dt} = \frac{Q(t) \cdot S(t)}{V\rho c} \quad (2)$$

Fig. 2 a, Real, dimensionless temperature amplitudes; b, phase angles.



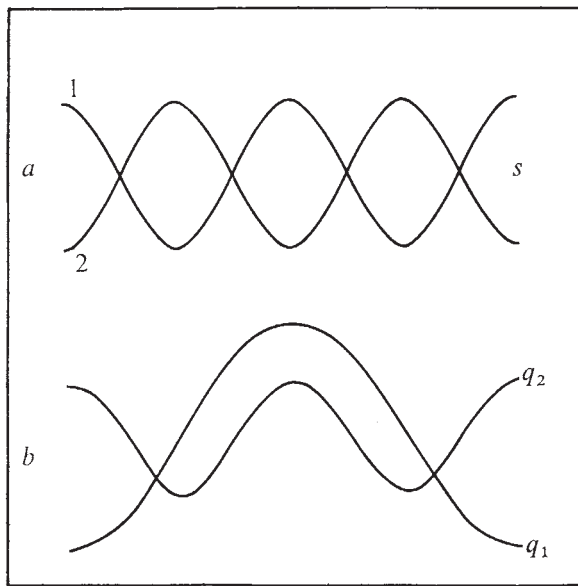


Fig. 3 *a*, Variation of surface area, s , during a day for two days separated by a time interval of seven days; *b*, variation during the day of the first and second harmonic constituents of solar radiation, q_1 and q_2 .

Let $S(t)$ be given by:

$$S(t) = S_0[1 + s_1 \exp(i\omega_2 t) + s_{-1} \exp(-i\omega_2 t)] \quad (3)$$

where $s_1 = s_{-1}^*$, and $2|s_1| < 1$

Without loss of generality $s_{1,-1}$ can be assumed to be real as long as $\omega_2 \neq n\omega_1$. We suppose ω_2 to represent the frequency of the semidiurnal lunar (M_2) tide. Equations (1)–(3) give:

$$\frac{dT}{dt} = \left[\sum_{n=-\infty}^{\infty} q_n \exp(in\omega_1 t) - kT \right] \times \left[1 + s_1 \exp(i\omega_2 t) + s_{-1} \exp(-i\omega_2 t) \right] / hpc \quad (4)$$

where $h = V/S_0$ denotes the effective mean depth of volume V . Equation (4) is a first order non-homogeneous differential equation for T with a periodic coefficient of T and a driving term containing the frequencies $n\omega_1$ as well as their sums and differences with ω_2 . We shall focus attention on the first and second harmonics of the daily frequency and their interaction with the tidal frequency, ω_2 , as far as these interactions have frequencies smaller than ω_2 . Thus, only the frequencies ω_1 , $2\omega_1$, $\omega_2 - \omega_1$, $2\omega_1 - \omega_2$ are considered here, to which respectively the complex amplitudes T_1 , T_2 , T_{12} and T_{22} are ascribed. The latter can be obtained by inserting in equation (4):

$$T = T_1 \exp(i\omega_1 t) + T_2 \exp(2i\omega_1 t) + T_{12} \exp(i(\omega_2 - \omega_1)t) + T_{22} \exp(i(2\omega_1 - \omega_2)t) + \text{complex conjugates} \quad (5)$$

and collecting terms with the same frequency. It is necessary to introduce several other quantities.

- $\tau = hpc/k$, a relaxation time^{2,3} representing the thermal inertia of a water mass.
- Dimensionless frequencies $\sigma_1 = \omega_1 \tau$ and $\sigma_2 = \omega_2 \tau$.
- The frequency ratio $r = \omega_2 / \omega_1$ ($1 < r < 2$).
- Dimensionless real temperature amplitudes

$$\theta_1 = k|T_1/q_1|, \theta_2 = k|T_2/q_2|, \theta_{12} = k|T_{12}/s_1 q_1|, \theta_{22} = k|T_{22}/s_2 q_2|.$$

● Phase angles: $\varphi_1 = \arctan(Im[T_1]/Re[T_1])$ and φ_2 , φ_{12} and φ_{22} . The solution for the θ s and φ s can be expressed in σ_1 and r and read, in first approximation:

$$\theta_1 = (1 + \sigma_1^2)^{-1/2}, \quad \varphi_1 = -\arctan \sigma_1$$

$$\theta_2 = (1 + 4\sigma_1^2)^{-1/2}, \quad \varphi_2 = -\arctan 2\sigma_1$$

$$\theta_{12} = \sigma_1 [1 + \sigma_1^2] \{1 + \sigma_1^2 (1-r)^2\}^{-1/2}$$

$$\varphi_{12} = -\arctan \frac{1 - \sigma_1^2 (1-r)}{\sigma_1 (2-r)}$$

$$\theta_{22} = 2\sigma_1 [1 + 4\sigma_1^2] \{1 + \sigma_1^2 (2-r)^2\}^{-1/2}$$

$$\varphi_{22} = -\arctan \frac{1 - 2\sigma_1^2 (2-r)}{\sigma_1 (4-r)}$$

These functions are shown in Fig. 2*a* and *b* for $r = 1.932$, corresponding to the ratio of the tidal (M_2) frequency and the daily frequency. As k may vary³ between 10 and 60 W m⁻² K⁻¹ and, for a tidal-flat sea, $0.1 < h < 10$ m is a representative interval, the range of interest for σ_1 will be $10^{-1} - 10^2$.

The components with the daily frequency and its second harmonic (θ_1 , φ_1 and θ_2 , φ_2) are the same as in the case of a water mass with a stationary surface area^{2,3}. For $\sigma_1 \rightarrow 0$, $\theta_1 \rightarrow 1$ and $\theta_2 \rightarrow 1$, whereas $\varphi_1 \rightarrow 0$ and $\varphi_2 \rightarrow 0$, indicating that for increasing exchange factor k or decreasing depth or frequency the instantaneous water temperature approaches its instantaneous equilibrium temperature, defined by $Q(t)/k$, as it ought to be for a water mass with a very small thermal inertia.

The temperature fluctuations caused by the interaction of the daily heat balance and the tidal cycle have a maximum, however, if $\sigma_1 = 1.0$ and $\sigma_1 = 2.5$. In other words, if the relaxation time, τ , is of the same order of magnitude as the daily period. In contrast to the amplitudes for the daily frequency and its higher harmonics, the interaction amplitudes tend to zero for $\sigma_1 \rightarrow 0$. This should be so, as for a water mass which approaches at any moment its equilibrium temperature the variation of its surface area does not matter.

If ω_2 represents the M_2 tidal frequency, frequencies $\omega_2 - \omega_1$ and $2\omega_1 - \omega_2$ are, respectively, 6.780×10^{-5} and 4.925×10^{-6} rad s⁻¹. Therefore, one of the consequences of the interaction will be a long-period temperature variation with a frequency of $2\omega_1 - \omega_2$ or a period of about 14.76 d. This will be observed as a variation of the daily mean water temperature.

The other interaction frequency ($\omega_2 - \omega_1$) corresponds to a period of about 25 h 42 min, somewhat larger than the daily period. As a consequence of the summation of trigonometric functions with slightly different periods, the sum of the temperature fluctuations with daily frequency and interaction frequency ($\omega_2 - \omega_1$) will be observed as a beat in the amplitude of the daily temperature cycle with a period of about 14.76 d. This can be seen as follows. If for $\exp(i(\omega_2 - \omega_1)t)$ is written $\exp(-i(2\omega_1 - \omega_2 - \omega_1)t)$ we get for the daily temperature cycle:

$$\{T_1 + T_{12} \exp[-i(2\omega_1 - \omega_2)t]\} \exp(i\omega_1 t) + \text{complex conjugate}$$

Thus, the amplitude of the daily cycle reads:

$$2|T'| = 2\{T_1 T_1^* + T_{12} T_{12}^* + T_1 T_{12}^* \exp[i(2\omega_1 - \omega_2)t] + T_{12}^* T_1^* \exp[-i(2\omega_1 - \omega_2)t]\}$$

which is simply an amplitude varying with frequency $2\omega_1 - \omega_2$ or with a period of about 14.76 d.

The phenomenon of the interaction of the daily heat balance and the tidal cycle can be understood from Fig. 3. Here, for two days separated by a time interval of seven days, the variation of surface area (s) because of the M_2 tide is shown together with the first and second harmonic constituents of the solar radiation (q_1 and q_2). In case 1 the maximum and minimum of

Table 1 Real temperature amplitudes ($^{\circ}\text{C}$)

h (cm)	$2 T'_1 $		$2 T_2 $	$2 T_{22} $
	max	min		
10	3.62	3.15	1.06	0.33
50	1.39	1.13	0.25	0.40
100	0.73	0.56	0.13	0.39
250	0.37	0.24	0.08	0.29
500	0.18	0.14	0.03	0.19
1,000	0.09	0.07	0.02	0.10

q_1 coincide approximately with the maxima of the water surface, whereas in case 2 they occur at its minima. The consequence is a variation in the amplitude of the daily temperature cycle with a period of 14.76 d. As for the second harmonic, q_2 , the maxima coincide with the maxima of s in case 1 and with the minima of s in case 2. Thus, in the first case there is a daily mean input of energy from this harmonic constituent whereas in the second case there is a daily mean output of energy. This causes a fluctuation of the daily mean temperature with a period of 14.76 d too.

In order to illustrate the theory we give a numerical example (Table 1) showing the values of the different real amplitudes for different depths of a tidal-flat sea. The values chosen for the parameters are: $k = 50 \text{ W m}^{-2} \text{ K}^{-1}$, $2|q_1| = 3\pi |q_2|/2 = 200 \text{ W m}^{-2}$, $2|s_1| = \frac{1}{2}$.

The interaction effects have a magnitude (Table 1) which is certainly sufficient to be detected with relatively simple techniques of measurement and which are, possibly, not without interest for ecological problems.

Of course, the interaction of the M_2 tide with the semidiurnal solar tide (S_2) does give rise to a spring-neap tidal cycle in the tidal range, which has the same period as the variation of the daily mean temperature and of the daily amplitude, explained here by the interaction of M_2 tide and solar radiation. Our explanation remains valid if an S_2 tide is present in the variation of surface area. In that case, the S_2 tide produces an apparent change in amplitude and phase of the temperature variations with daily frequency and higher harmonics. The existence of an M_2 tide interacting with solar radiation remains necessary, however, to explain the appearance of the 'new' frequencies $\omega_2 - \omega_1$ and $2\omega_1 - \omega_2$, and, consequently of the varying daily mean temperature and amplitude.

Real situation

Until now, there have been no systematic surveys of temperature fluctuations in tidal-flat seas. We have made extensive measurements in the Dutch Wadden Sea, which is part of an estuarine inshore area along the Dutch, German and Danish coasts, separated from the North Sea by a chain of islands. An important part of the area called 'Wadden', emerges at low tide as muddy sand flats. A typical part of this tidal-flat sea is the Mok bay, situated at the southern tip of Texel Island (Fig. 4). A tidal channel connects the bay with the western part of the Wadden Sea. Through this channel water which covers the tidal flats flows in and out with the tide. The mean volume of the bay is about 10^6 m^3 ; the area of the tidal flats, including the tidal channel, amounts to $8 \times 10^5 \text{ m}^2$. The tide in this area is dominated by the M_2 tidal cycle. There is also an obvious spring-neap tidal cycle. The latter, however, has only a minor effect on the fluctuation of the surface area as, because of the geometry of the bay, high tide surface area and low tide surface area are approximately the same at spring tide and at neap tide.

In the tidal channel (Fig. 4b) the temperature was recorded at three different depths, at intervals of 5 min. For the purpose of this study, records taken at intervals of 15 min were sufficient. On that basis, the daily mean temperature was calculated. At one of the tidal flats all of the relevant meteorological parameters were recorded; these included net radiation, air temperature, humidity and wind velocity. The accuracy of water temperature measurements was checked with reversing thermometers, and

seemed to be 0.1°C . Measurements were carried out over three periods: April 21–May 5, 1972; August 22–September 21, 1973 and June 21–August 29, 1974. Thus, about 110 days of records were available for analysis. As is to be expected in this area, daily mean values and daily amplitudes of water temperature varied greatly. To demonstrate that the records contain periodicities induced by the tide, as predicted by theory, 6 periods, each covering 15 d, were considered.

To separate short-term variations of the daily mean water temperature, including tidal effects, from background, long term fluctuations, such as increases or decreases of water temperature caused by seasonal heating or cooling, the daily mean difference between the temperature of the channel and that of the adjacent North Sea was calculated. For the latter, water temperature measurements taken from the lightship Texel (Fig. 4), positioned about 20 miles from Mok Bay, were used. These are thought to be influenced by long term meteorological processes, but not by the effects of the tidal flats.

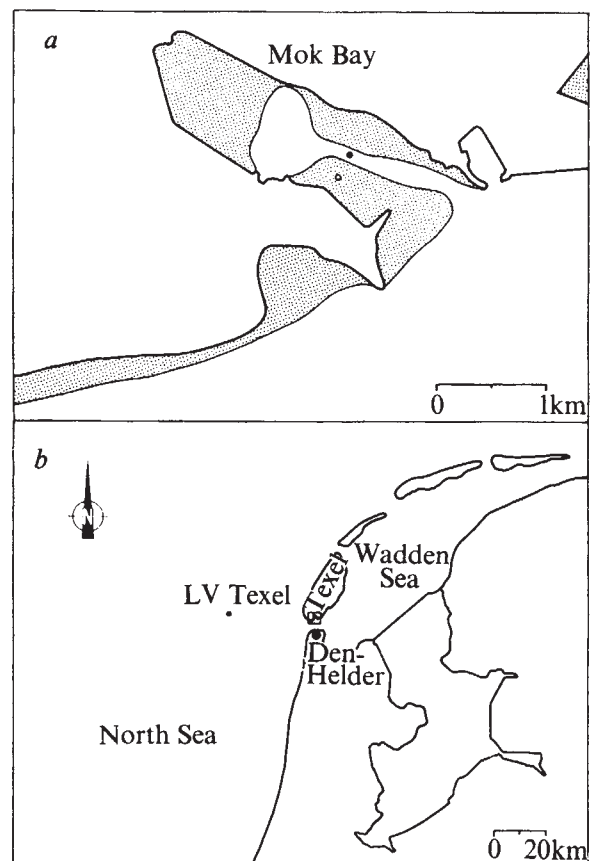
Next, for each of the six periods, a smoothed curve of the temperature differences was obtained using the formula: $\bar{X}_i = \frac{1}{4}(X_{i-1} + 2X_i + X_{i+1})$, where $\bar{X}_i$ is the smoothed value for the i th day and X_{i-1} , X_i , X_{i+1} are daily mean values.

Then, for each of the six periods a straight line was drawn through the smoothed values by least-squares fit, to account for any trend during the period of 15 d. For each day in a particular period the deviation from this line was taken.

Finally, the last values were normalised for each period by dividing through the mean of the absolute values of the positive and negative extreme deviation from the least-squares-fit line.

After normalising the six periods, an average curve for each 15-d period was constructed in such a way that days having high tides at approximately the same time as one another occupied the same place in the sequence. The resulting curve, dimensionless by normalising, was transformed back to daily deviations in $^{\circ}\text{C}$, from the period mean.

Fig. 4 Situation of the Mok Bay on large (a) and small (b) scale. The former shows the points of registration of meteorological parameters ($\circ$) and water temperature ($\bullet$).



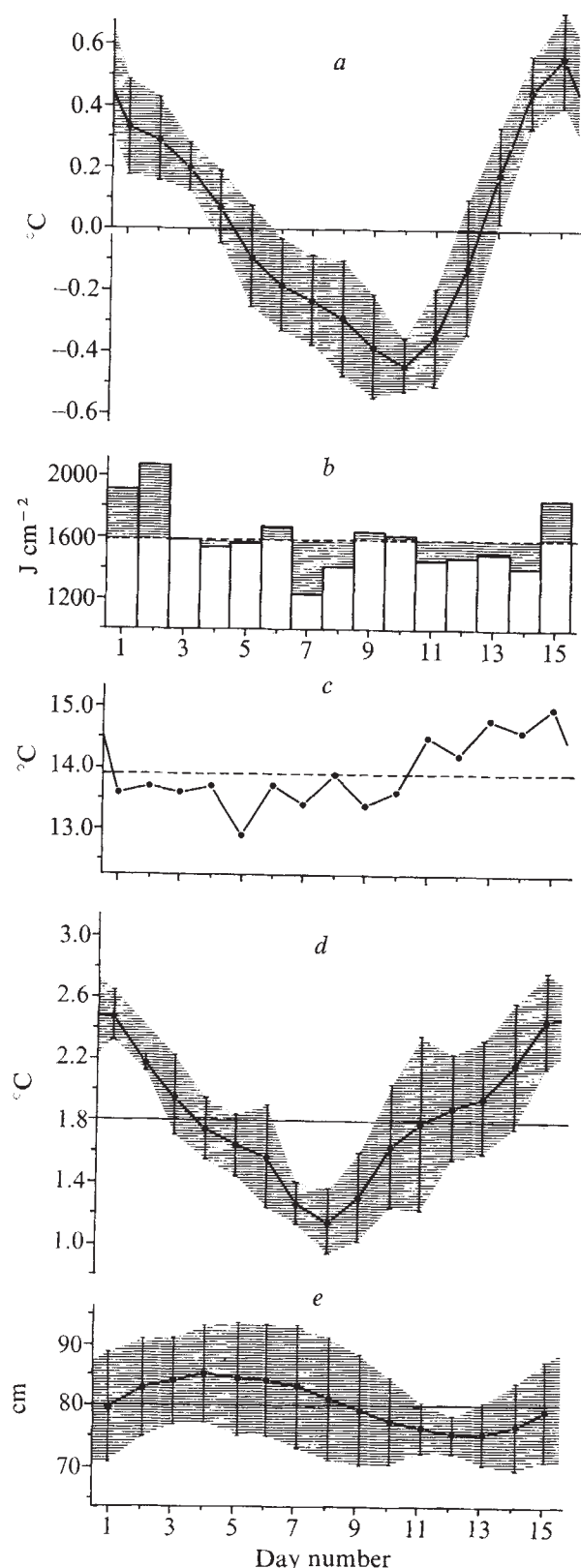


Fig. 5 a, Average curve of the deviation of daily mean water temperature of Mok Bay from its mean value over a 15-d period, showing also standard-deviation bars; b, average variation of global radiation over a sequence of 15 days; c, average curve of the daily mean air temperature; d, average curve of the difference between daily maximum and minimum water temperatures of the Mok Bay, showing also standard-deviation bars; e, average curve of high tide level in the Mok Bay over a sequence of 15 days; bars denoting the standard deviations.

The result is shown in Fig. 5a. There is a significant difference between the temperature deviation curve and any straight line and the curve agrees well with the predicted periodicity of the daily mean water temperature in a tidal-flat area.

To show that this result is not caused by an accidental variation of the energy input (q_0), having the same period, average values of global radiation, which constitutes the most important part of q_0 , were calculated. For this we have taken values measured at Den Helder (Fig. 4a) by the Royal Netherlands Meteorological Institute. The mean global radiation for the 90 d used in the analysis is $1,585 \text{ J cm}^{-2} \text{ d}^{-1}$. The average deviation for each day of the 15-d periods is shown in Fig. 5b. It is clear that there is no correlation between the variations in global radiation and the curve of Fig. 5a. For the same purpose this procedure was carried out for the air temperature. The mean air temperature for the 90 d, observed at land station Den Helder, supposed not to be influenced by any tidal effect, was 13.9°C . The air temperature curve averaged over six periods is drawn in Fig. 5c. As for the global radiation, there is no apparent correlation between the average daily air temperatures and the curve of Fig. 5a.

The asymmetrical behaviour of the curve of Fig. 5a could be explained by the fact that, in this area, the moment of high tide itself shifts in an asymmetrical way during a 15-d period. Of course, part of the asymmetry may be caused by a variation in the atmospheric background, which has not been removed by averaging over only six periods.

As predicted theoretically, the daily amplitude varies with a period of about 15 d as well. As the maximum and minimum water temperatures are strongly dependent on weather conditions, the procedure used here to calculate the daily mean water temperature was used, without the first step and with a more extensive smoothing formula⁴:

$$\bar{X}_t = \frac{1}{35} [17X_t + 12(X_{t+1} + X_{t-1}) - 3(X_{t+2} + X_{t-2})]$$

To obtain the daily maximum and minimum water temperatures the mean values of the 5 highest and of the 5 lowest values of the daily records were taken (Fig. 5d). From this figure it may be concluded that the daily amplitude shows a periodic behaviour in agreement with theoretical prediction.

There is no need to regard the spring-neap tidal cycle as responsible for the results presented here, rather than the interaction of the M_2 tide and solar radiation. To illustrate this, we have shown (Fig. 5e) the average curve of the high tide level in the Mok Bay during a 15-d sequence. There is indeed a slight spring-neap tidal cycle visible. Note, however, the large standard deviations which are caused partly by influences on water level other than those of the astronomical tides. The figure demonstrates, at least, that the maximum of the daily amplitude does not occur at spring tide and that the minimum does not coincide with neap tide. The same applies to the comparison of Fig. 5a with Fig. 5e.

The variation of the daily mean water temperature is about 0.9°C , whereas the daily amplitude varies from 0.6 to 1.2°C . These values are not in agreement with Table 1 if the mean depth is assumed to be about 100 cm . The numerical disagreement is probably caused by the great difference in geometry between the idealised theoretical model and the complicated tidal-flat area of the Mok Bay. Moreover, in theory a well mixed basin was assumed, whereas in reality temperature distribution in the Mok Bay is not uniform. Consequently, advection by tidal currents will affect the temperature variation, influencing especially the daily amplitude. None the less, it may be stated that a qualitative agreement exists between theory and experiment.

Received October 29, 1974; accepted March 10, 1975.

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Long-range transport of photochemical ozone in north-western Europe

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Measurements of the concentrations of ozone and trichlorofluoromethane in the atmosphere over various parts of the southern British Isles provides evidence to suggest that photochemical ozone and the pollutants from which it is formed may be transported into the region from their origins in continental Europe. It seems likely, therefore, that even isolated areas, away from centres of industrialisation, may suffer the effects of this pollution.

THE concentration of ozone in air at ground level increases during sunny, anticyclonic weather¹⁻³. The maximum hourly concentrations of 0.11–0.12 p.p.m. are 2–3 times as high as expected in unpolluted air near to the ground. This suggests that photochemical ozone is widespread and is not confined to localities in which pollutants such as oxides of nitrogen and hydrocarbons are emitted.

In 1973 the ozone concentration was measured at three widely spaced locations on an E–W line across southern England and Ireland. The sites (Table 1) were in rural areas away from large, local sources of pollution and also away from forested areas and other sources of natural hydrocarbons. The most remote site, at Adrigole, was chosen in the expectation that it would provide a measure of the 'background' level of ozone concentration for the maritime regions of north-western Europe. Meteorological data were obtained from nearby meteorological stations (Table 1). In addition, local meteorological conditions at Harwell were recorded on an automatic weather station at the sampling site.

The ozone concentration was measured continuously using ethylene-chemiluminescence monitors. Ambient air was sampled at a height of 2 m above ground through polytetrafluorethylene tubing. A filter was used to prevent the entry of particulate matter into the detector and flow-control systems. The detectors were calibrated using a constant ozone source standardised with neutral-buffered, potassium iodide solution⁴. Regular checks on the calibration of the instruments at Harwell and Sibton revealed no significant drift in the zero or full-scale settings over the period of measurement. Routine calibration checks on the Adrigole instrument could not be made. The net change in sensitivity was, however, less than 10% over 5 months. Any zero drift was corrected by automatically recording the photo-multiplier dark current once every hour.

The concentrations of halogenated hydrocarbons as well as of ozone were measured at Adrigole. Air samples were taken twice

daily, at 0500 and 1400, on an automatic gas chromatograph equipped with an electron capture detector operating in the coulometric mode⁵. The chromatograms were recorded on a strip chart from which absolute concentrations of CCl₃F and CCl₄ were determined at a later date. Similar measurements were made at Bowerchalke, Wiltshire (90 km south-west of Harwell) and using those results in conjunction with the Adrigole measurements we obtained information on the history of air masses over the southern British Isles at any given time. The trajectories of air masses were computed backwards in time for Adrigole and Sibton. These air trajectories were based on mean winds, computed for the layer 300–2,000 m above the average terrain using a Northern Hemisphere radio sonde observation data set. Each wind vector in the area around the point of interest was weighted by $1/R^2$ where R is the distance between the observing station and the point of interest. A 'central tendency' extrapolation scheme was used to trace the 'air' back in time.

Table 1 Latitudes and longitudes of sampling locations

Sampling location	Position	Meteorological stations
Adrigole, County Cork, Ireland	51°40'N 9°45'W	Valencia
Harwell, Oxfordshire, England	51°35'N 1°19'W	Heathrow
Sibton, Suffolk, England	52°03'N 1°05'E	Gorleston

Trajectories for all four synoptic times (0000, 0600, 1200, and 1800 GMT) were constructed, but only those for midday (1200 GMT) are considered here. The trajectories for Adrigole were carried backwards in time for a maximum of 5 d (120 h) which usually complied with the prescribed space limitations for wind vector interpolation.

At Sibton, it was possible to estimate the trajectories up to a maximum of 10 d (240 h) before the arrival of the 'air'.

Results and discussion of daily measurements

The maximum hourly mean ozone concentrations at the observation stations during each day are shown in Fig. 1. The concentrations of CCl₃F at Adrigole and Bowerchalke are also shown. CCl₃F is an inert anthropogenic gas, and its concentration gives an index of pollutants in an air mass.

Ozone. Table 2 gives the frequency distribution of maximum hourly mean concentration of ozone. The range (0.03–0.14 p.p.m.) was similar at all three sites and, surprisingly, the number of days on which the US standard⁶ of 0.08 p.p.m. was exceeded at Adrigole (17 days) was similar to that at Harwell

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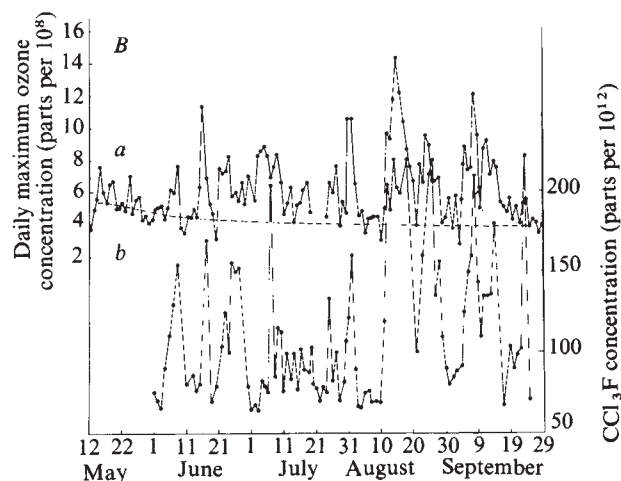
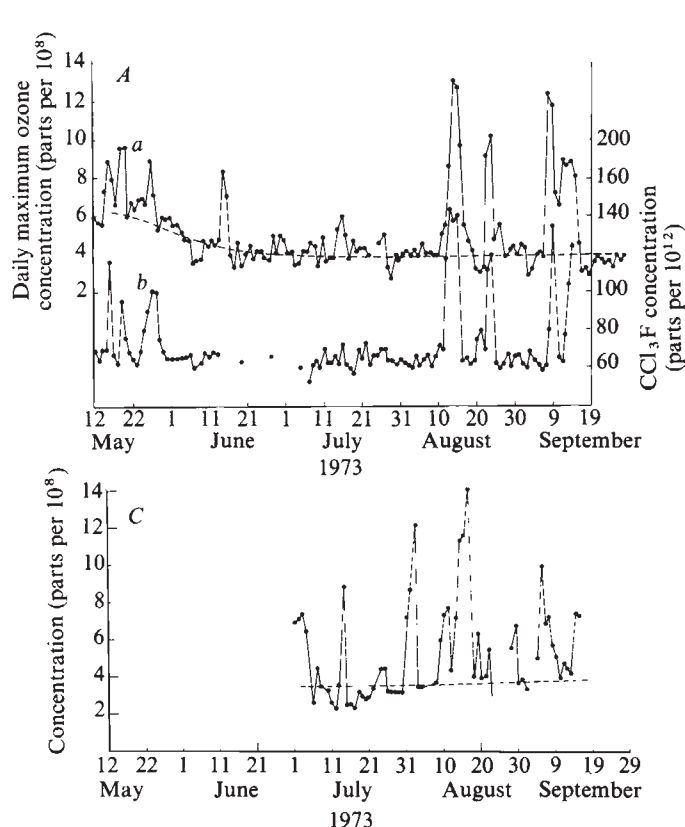


Fig. 1 Daily maximum ozone concentrations and CCl_3F concentrations. A, Adrigole, Southern Ireland: a, ozone; b, CCl_3F . B, a, Ozone from Harwell, Oxfordshire; b, CCl_3F from Bowerchalke, Wiltshire. C, Ozone from Sibton, Suffolk.

(23 days) although Adrigole is much further from sources of the precursor pollutants than Harwell.

Incidents of elevated ozone concentration superimposed on a 'background' level (Fig. 1) are clearly indicated at each site. We believe this 'background' level represents the natural ozone content of the lower troposphere over north-western Europe.

The mean background concentration at Adrigole was about 0.04 p.p.m. compared with 0.03 p.p.m. at Mauna Loa and 0.02 p.p.m. at Point Barrow, Alaska (both in September, 1973).

The pattern of the incidents, particularly during August and September was similar at each of the three sites.

Fluorocarbons. The general pattern of the fluorocarbon concentrations was very similar to the maximum hourly mean ozone concentration data—values varied in southern England but stayed near background level for longer periods at Adrigole. The background concentration of CCl_3F at Adrigole was 68 parts per 10^{12} by volume (calculations were based on concentrations observed when the air masses were coming from the Atlantic). In polluted air, concentrations of up to 370 parts per 10^{12} by volume were observed. Figure 1 shows clearly that ozone and CCl_3F were advected in polluted air over south-western Ireland. Comparisons of ozone concentrations at Harwell with CCl_3F concentrations at Bowerchalke shows the same thing.

The correlation coefficient between CCl_3F and O_3 at Adrigole was 0.74, based on measurements taken at 1400 GMT May–September, 1973. The higher values ($\text{CCl}_3\text{F} > 100$ parts per 10^{12} by volume; $\text{O}_3 > 0.1$ p.p.m.) were deleted and the correla-

tion recomputed, resulting in a reduced correlation coefficient of 0.49.

We conclude that a significant portion of the ground-level ozone in southern England during the summer is anthropogenic in origin and that in suitable meteorological conditions ozone and/or its precursors can be transported over distances of the order of 100–1,000 km, to give appreciable levels of pollution in locations far from the source.

Diurnal variation in ozone concentration

Figure 2 shows the diurnal variation of ozone at all three sites during August 11–18, 1973. Figure 3 shows the frequency distributions of the daily maxima as a function of time of day over the whole period of measurements at Harwell and Adrigole. At Harwell and also at Sibton, there were consistent daytime maxima and nocturnal minima. There was no marked diurnal variation at Adrigole. On several occasions maxima occurred during the night.

At inland stations shallow nocturnal inversions are common, particularly during anticyclonic weather in summer. Ozone is destroyed at the Earth's surface, and may also be removed by gas-phase reactions with other pollutants. Below an inversion, the downwards diffusion of ozone from above is inhibited, and thus the concentration can be expected to fall. During the day, convection is strong, more ozone is brought down, and the concentration rises. Adrigole is on a peninsula and is only

Table 2 Frequency distribution of maximum hourly average ozone concentration for the period May 12, 1973 to September 29, 1973

Maximum O_3 concentrations (parts per 10^8)	0–4.9	5.0–7.9	8.0–10.9	11.0 or greater	Data missing
	Number of days				
Adrigole	85	33	13	4	6
Harwell	51	60	18	5	7
Sibton	47	21	4	4	15
(July 1–September 29 only)					

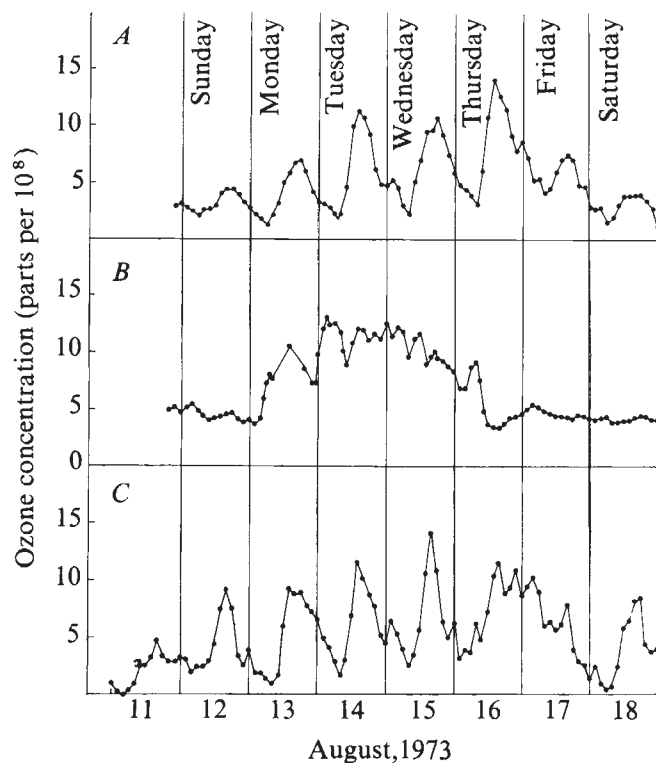


Fig. 2 Ground-level ozone concentrations during the period August 11–18, 1973. A, Sibton; B, Adrigole; C, Harwell.

400 m from the sea. At coastal locations, nocturnal inversions are less common. Moreover, ozone is destroyed more readily at land surfaces than at sea surfaces^{6–8}, thus, the difference in the patterns of diurnal variation between Adrigole and other sites can be explained. The diurnal pattern of photochemical ozone concentrations in urban areas has been attributed to the diurnal variation of insolation, with each day treated as an episode. Measurements at stations very near the coast may demonstrate the continuity of the photochemical process over several days.

Transport of ozone

During the period August 11–August 18, 1973 an anticyclone became established over the North Sea, replacing a south-westerly air flow from the Atlantic by a predominantly easterly flow of continental air over the southern British Isles. The stable weather, with nearly cloudless days was interrupted by the eastward passage of a weak cold front over the entire region during August 16 and 17, after which the air flow was predominantly westerly. The changes in air flow during the period are illustrated in the back-track air mass trajectories for Adrigole and Sibton (Fig. 4).

The ozone concentration data for August 11–18 (Fig. 2) show the presence of elevated ozone concentrations at all three sites by the time the arriving air masses had been in transit over the continent for at least 24 h. After the passage of the cold front and the advection of air directly from the Atlantic, the ozone concentrations declined again to near normal values.

Table 3 Trajectory length (km) and transit time from European continent to Adrigole

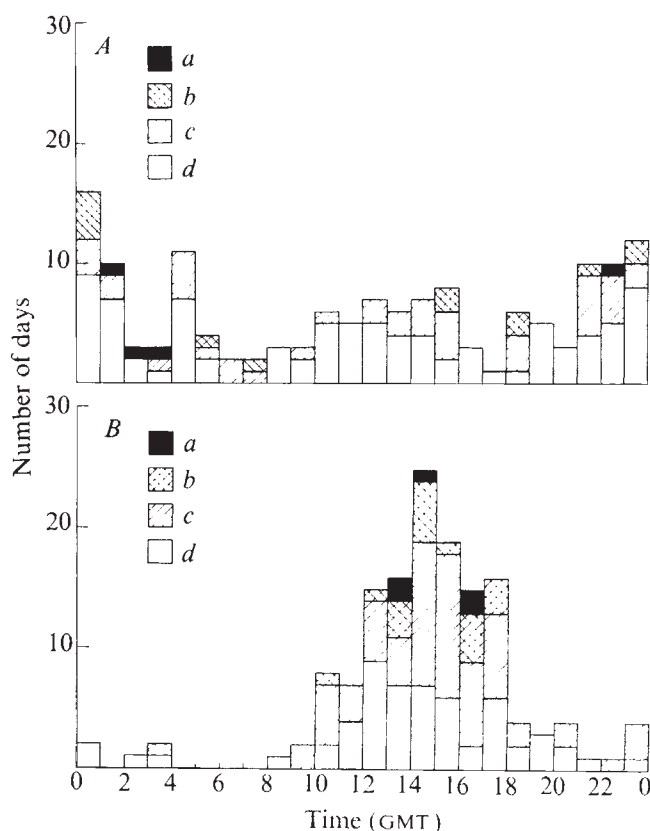
Date	Time	O ₃ (p.p.m.)	Length (km)	Time of transit (h)	Speed (km h ⁻¹)
13	1200	0.10	700	48	14.6
14	1200	0.12	700	33	21.2
15	1200	0.11	655	48	13.7
16	1200	0.05	1,310	96	13.7

It is clear that the elevated concentration of ozone observed during the episode at Sibton, situated 10 km from the Suffolk coast in sparsely populated country, must have had its origin in continental sources outside the UK. The trajectory and CCl₃F data also confirm that advection from the continent led to the high ozone concentration at Adrigole. Contribution from the UK was also negligible; the air mass trajectories, although less reliable than those at Sibton because of the paucity of observations nearby, back-track to the south-east, towards France. The sources of emissions giving rise to the high values at Harwell are less clear. The maximum levels observed at Harwell are, however, not significantly different from those at Sibton, Adrigole or even central London (0.136 p.p.m. on August 15, 1973) showing that by August 15 a sufficiently high concentration of pollutants had accumulated in air of continental origin to give similar elevated concentrations over much of southern Britain and Ireland, and probably over more extensive areas also. It is not possible, therefore, to distinguish the specific contribution to the total pollution of sources in the UK.

The data obtained at Adrigole show that elevated ozone concentration can be sustained in air masses several hundred kilometres from the sources of emissions, which in turn indicates a relatively long natural lifetime for ozone in the lower troposphere. The overnight persistence of the elevated ozone levels requires a lifetime of at least 10 h. Table 3 and Fig. 4 show that advection to Ireland required from 30–100 h.

Considering that the Adrigole values were approximately as high as those in England, this implies that the effective lifetime of ozone is of the order of 1–2 d. It is not possible from these limited data to disentangle the several factors controlling concentration (source strength, vertical dispersion, ultraviolet intensity, removal rates and so on). Table 3 shows, however, that the highest values at Adrigole on August 14, 1973 coincided

Fig. 3 Time of maximum hourly mean ozone concentrations during the period May 4–October 2, 1973. A, Adrigole; B, Harwell. In both cases: a, maximum concentration greater than 0.11 p.p.m.; b, 0.08–0.109 p.p.m.; c, 0.05–0.079 p.p.m.; d, 0.00–0.049 p.p.m.



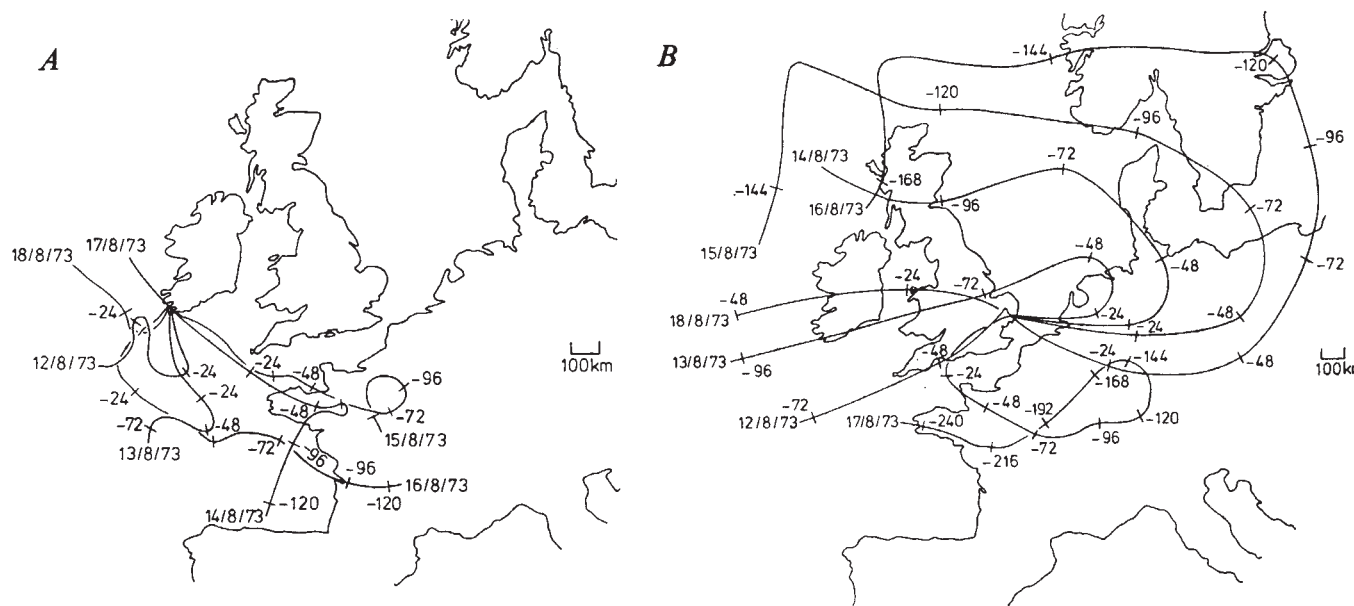


Fig. 4 Back-track air trajectories for 1200 GMT each day for Adrigole (A) and Sibton (B), together with position of air mass on preceding days.

with the shortest transit time over the sea; on August 16, however, the values, even before the frontal passage, had lowered by about 0.05 p.p.m. and were associated with trajectories and transit times twice those observed on August 14 and 15, 1973.

The alternative process of long range transport of pollutant precursors, with ozone formation occurring in response to local meteorology in each locality, is inconsistent with the high nocturnal ozone concentrations found at Adrigole. It would also require the formation of very similar ozone concentrations each day from what must be different precursor concentrations in Suffolk, central London, Oxfordshire and south-western Ireland.

We note, without comment on its relevance to the UK or Europe, that the US Air Quality Standard for photochemical oxidants was exceeded on a number of days during 1973 at all three sampling sites, including one situated in southern Ireland. We conclude that in suitable meteorological conditions measurable amounts of photochemical pollution of anthropogenic origin are transported over distances up to at least 1,000 km in north-western Europe and that on occasions continental emissions provide a major contribution to photochemical pollution in the UK. During such periods photochemical pollution is essentially a regional rather than local problem with uniformly elevated concentrations of photochemical ozone being observed simultaneously over different parts of

the UK. It follows that, should controls on the emission of the pollutant precursors (hydrocarbons and nitrogen oxides) be considered necessary in order to reduce future levels of photochemical oxidants in the UK, then such controls are unlikely to be effective in the absence of similar controls elsewhere in Europe.

We are indebted to Mr Michael O'Sullivan and to the staff of Blyth Rural District Council, who maintained the monitoring equipment at Adrigole and Sibton, respectively. We thank the East Anglian Water Company for permission to use the Lodge Wood Water Tower, Sibton. Air mass trajectories were prepared by Mr J. Heffter of the Air Resources Laboratory. One of us (J. E. L.) thanks Shell Research Ltd. and the Manufacturing Chemists Association for financial support for part of this work. The ozone measurements at Sibton, Harwell and Adrigole were carried out as part of a programme of atmospheric pollution research sponsored by the UK Department of the Environment.

Received January 20; accepted March 27, 1975.

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Construction of bacterial flagella

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The flagella of Salmonella can adopt a number of distinct helical forms. This article discusses the design of a subunit which packs to give a helical filament. Polymorphism is explained by small changes in the geometry of the subunit.

BACTERIAL flagella are constructed from molecules of the protein flagellin by a process of self-assembly¹⁻⁶. It is easy to see how a straight filament can be polymerised in this way, with the subunits arranged in a regular array in identical environments, as for example in tobacco mosaic virus⁷; but bacterial flagella in general have a helical shape, and it is hard to see how this can

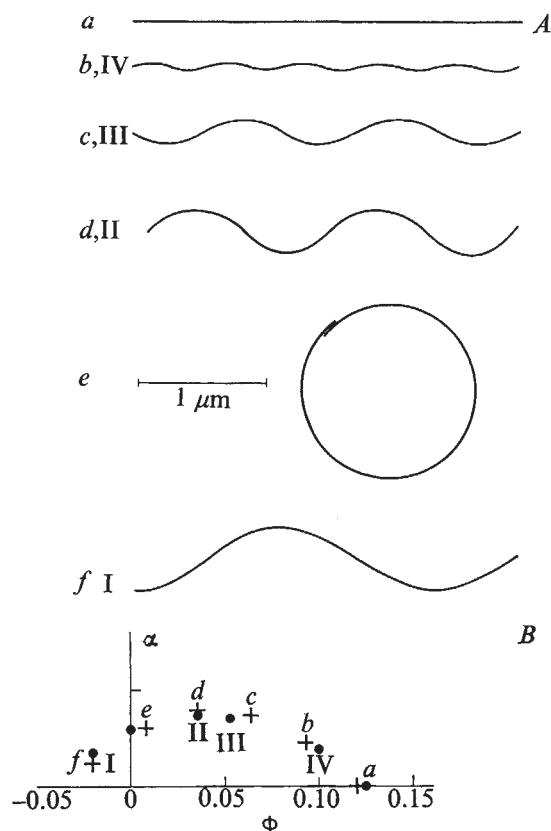


Fig. 1 *A*, Distinct helical forms made by copolymerisation of monomers *SJ 670* and *SJ814* (after Asakura^{8,9}). Roman numerals are Asakura's identification, but lower case letters (*a-f*) are used for the corresponding theoretical classes proposed in this paper. Form *e* is circular, or closecoiled. Monomer *SJ670* can build *e* or *f*, and *c* and *d* if the pH is changed; and monomer *SJ814* can build *a* or *b*. Copolymerisation produces forms *b*, *c* and *d*. The thickness of the filaments is roughly to scale. *B*, Curvature-twist plot for the six filaments shown in *A*. ●, Mean values from Asakura's^{8,9} data (but twist of straight filament from O'Brien and Bennett¹¹). +, Proposed model.

be achieved by bonding identical units to each other in a regular manner³. Furthermore, flagella of various strains of *Salmonella typhimurium* adopt, in different environments, a number of distinct polymorphic forms with different helical parameters^{8,9}.

I attempt to resolve this problem by designing a building block, within the framework of classical mechanics, which will assemble in quantity into a helical filament. This unit connects to six neighbours: four at an outer level, near the surface of the filament, and two at an inner level. When connected only by the outer contacts, the units can assemble into a family of distinct helical waveforms which correspond closely to those observed. The function of the inner connections between units is to select a particular member of the family. The model predicts a simple relationship between the total number of polymorphs and the number of longitudinal rows of subunits in the helical surface lattice.

Selected summary of present knowledge

The surface lattice of a bacterial flagellum is made up of a number of helical families, of which the most important are one-, five-, six- and eleven-start helices (Fig. 2*a*). This lattice is common to filaments from all strains so far investigated¹⁰⁻¹². The main facts about the waveforms of the filaments have been reviewed by Asakura⁸ and Asakura and Iino⁹. Figure 1*A* shows the distinct waveforms observed when flagella are constructed *in vitro* from monomer obtained from strains *SJ670* and *SJ814*, both separately and mixed^{8,9}.

From measurements on the waveform of a given filament it is possible to deduce, by simple geometry, the uniform twist and curvature which must be imported to the filament by the local building process. Here twist is described by the inclination ϕ (radians) of the longitudinal helices in the surface lattice to the local axis of the filament, and curvature α by the (percentage) difference in length between the longest and shortest of these longitudinal columns; I take the diameter of the filament, nominally, as 20 nm. It is useful to describe the results in the form of a plot of curvature against twist; Fig. 1*B* shows the data for the waveforms of Fig. 1*A*. The diagram incorporates recent observations¹³ on the helical handedness of various waveforms. The circular filament has zero twist. In the case of a

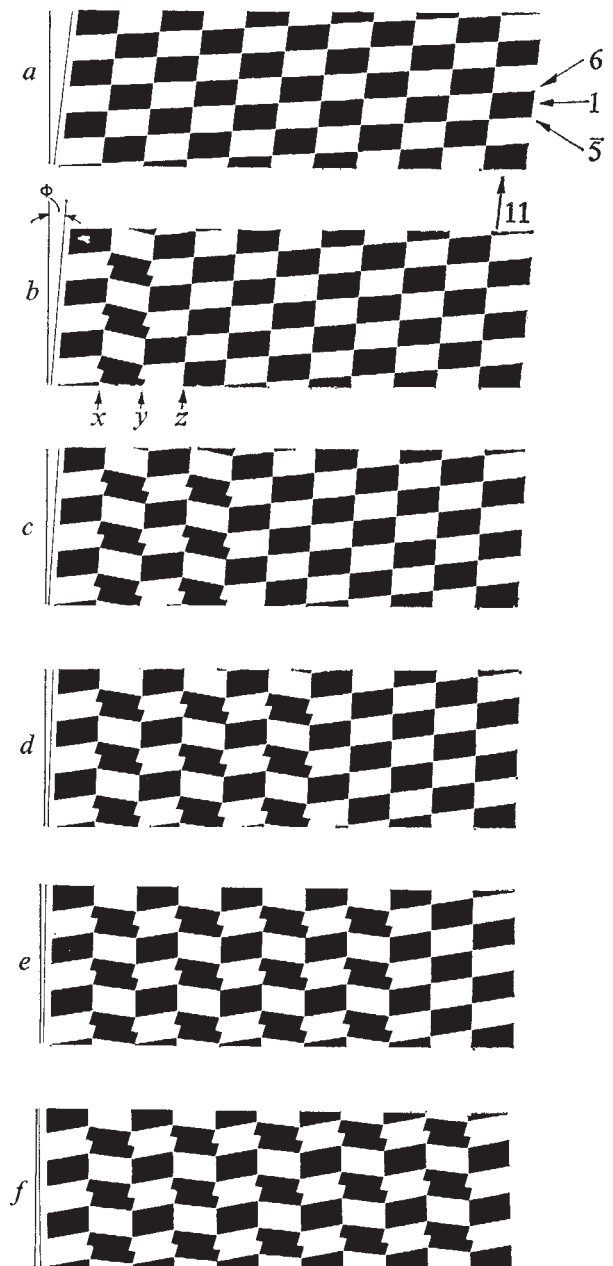


Fig. 2 Surface packings corresponding to the six ideal waveforms (*a-f*) viewed from the outside. The lower and upper edges of the patches each form a circle when the packings are wrapped into a cylindrical surface. A member of the one-, five-, six- and eleven-start helices in the surface lattice is picked out in *a*. Longitudinal (eleven-start) lattice lines make angle ϕ with the local axis; these two directions are shown at the left. Curvature is produced by small differences in axial length between 'strands', that is, lines of contacts, *x*, *y*, *z*, marked in *b*. Inner connections between units are not shown.

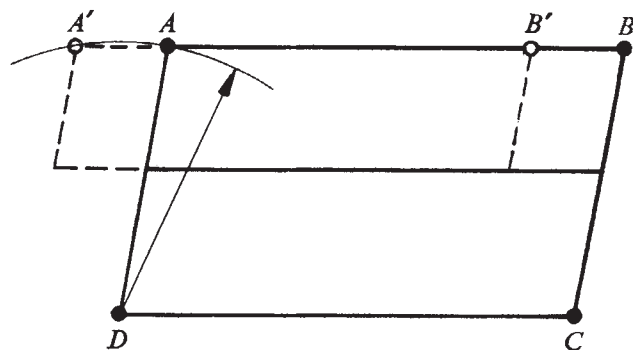


Fig. 3 Layout of connecting points on flat units in Fig. 4. ●, Normal connections; ○, alternative connections. The unit has two stable configurations involving relative motion of two parts of the unit, which are connected by interior bonds (not shown) having a bi-stable feature. The units fit exactly in the plane arrays of Fig. 2, but the design could easily be altered so that $A'D$ and $B'C$ are slightly less than AD and BC respectively.

straight filament the curvature is zero, and the twist can only be found from a knowledge of the surface lattice; O'Brien and Bennett¹¹ have found the twist of straight filaments of *SJ814* by high resolution electron microscopy using optical filtering techniques¹⁴.

Surface packings and unit design

Consider the surface packings of identical flat units shown in Fig. 2. In *a* the interunit connections are at the corners of the quadrangular units, giving a uniform packing, but in *b-f* units in some columns have undergone a change in configuration which makes them rotate forwards. This change is here represented as a shear in the tetragonal disposition of the bonding sites (Fig. 3). The effect of rotation of units in a column is to shear the lattice along a nearly axial line of units, thereby altering the tilt of the columns with respect to the base-line, and thus changing the twist of the assembly. Each column so rotated gives an additive contribution to the twist of the assembly. A special feature is that rotated columns are clustered but are not adjacent to each other; I shall explain this later.

Now the two configurations of the unit shown in Fig. 3 are arranged so that the longitudinal dimensions are the same, that is, $A'D = AD$ and $B'C = BC$, and the units in the plane arrays of Fig. 2 fit exactly. It would be easy, however, to arrange for these dimensions to be slightly different, so that a longitudinal strand of connections such as *x* or *y* (Fig. 2*b*) is slightly shorter (say) than a strand *z* of regular bonding. If in addition the units are given a degree of longitudinal elasticity the units will assemble, in the round, to a curved filament. Indeed, with units having these features it is easy to show by the methods used for analysing thermal stress in elastic structures¹⁵ (the difference in unstressed lengths of the strands being analogous to thermal expansion) that the curvature of filaments built according to the six patterns *a-f* is proportional to $\sin 2n\pi/11$, where *n* is the number of rotated columns, whereas the twist (relative to the twist for pattern *a*) is proportional to *n*. Hence a plot of curvature against twist gives a sinusoidal, discrete variation (Fig. 1*B*).

If we assign a reasonable degree of tolerance for the distortion of the filaments in the course of preparation for microscopy^{8,9}, we can see that the surface states *a-f* in Fig. 2 correspond closely to the waveforms observed by Asakura. This correspondence forms the basis of my proposed model.

Features of the surface packing

There are several crucial features of the packings shown in Fig. 2, without which the model would not work. First is the ability of the units to adopt two stable attitudes. The unit of Figs 2 and 3 has an internal movement controlled by a bi-stable 'switch' analogous to that already described¹⁶ at the α - β interface in a haemoglobin molecule.

Second, each unit is connected to only four neighbours. A coordination number greater than this would inhibit the mechanism involved in changing between states in the surface arrays. This follows from the kinematics of interconnected rigid bodies: a full description will be given elsewhere.

Third, since the packings involve a chequerboard pattern, the longitudinal length of the assembly is determined by the strands of connections between adjacent columns of units (for example, *x*, *y*, *z* in Fig. 2*b*) rather than the individual columns themselves. This accounts (see next section) for some special features.

The notions that there are two inherently stable conformations of the subunit, and that different helical waveforms correspond to different numbers of longitudinal columns in the two states have been put forward by Asakura⁸, and elaborated by Wakabayashi and Mitsu¹⁷ who indeed did an elastic calculation on the curvature resulting from different numbers of columns in an alternative configuration. The absence of detailed consideration of interelement bonding from this analysis¹⁷ (my crucial features two and three) however, prevented them from taking their model beyond a 'phenomenological' stage.

Why do 'rotated' columns cluster?

I have explained that the difference in the dimensions AD and BC between the two alternative configurations of the units gives a shortening of strands on one side of the filament and consequently a curvature. There is in fact a stringent geometrical requirement that the relative lengths of the strands of connections should vary sinusoidally around the circumference (Kirchoff's hypothesis¹⁸). Consequently each strand must be distorted a little from its natural long or short configuration to make it fit (except in case *a*). There will thus be an elastic strain energy of distortion¹⁵ associated with any given packing, and it is easy to show quantitatively that, for a given number of columns in the alternative configuration, the strain energy is least when they are clustered precisely as shown in Fig. 2*b-f*. Figure 4 shows an example of this. The minimum is deep, and it is reasonable to suppose that this is why other packings are excluded.

Inner connections between the units

The outer connections described so far enable the units to assemble in regular packings like those of Fig. 2*a* but having an

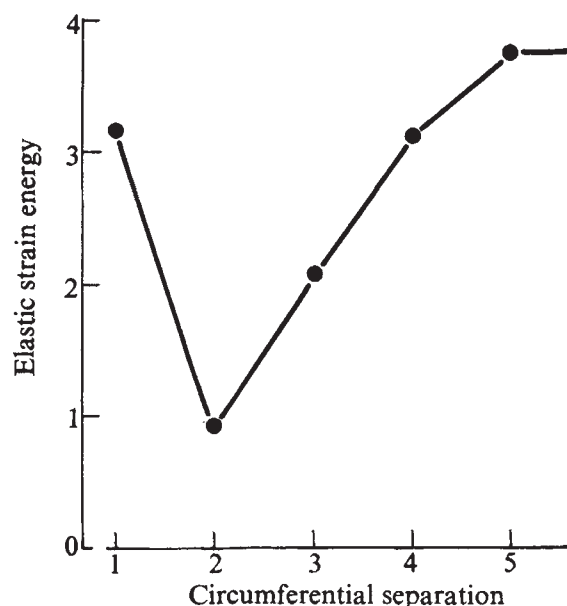


Fig. 4 Strain energy of distortion of a packing involving two columns of rotated units, disposed in various ways. Circumferential separation is counted in numbers of columns; a value of 1 means that the columns are adjacent, and so on. Arbitrary but consistent units of elastic strain energy.

arbitrary number of longitudinal columns around the circumference. We can make the packing contain just 11 columns by insisting that adjacent units make an angle of $360^\circ/11 \approx 33^\circ$ when viewed along the local axis. This can be done in various ways, all involving inward-facing projections on the units, with interunit connections at the tips. In fact the angular rotation of consecutive units on one of the six-start lattice helices (say) is slightly different for each of the six packings shown in Fig. 2, varying from 34.7° in *a* to 32.4° in *f*. Thus a fine control of this angle, by small changes in conformation of the inward projections, can determine which of the packings is to be built: a wrong packing would not allow simultaneous bonding between the inner and outer structure of the assembly³.

Design difference in flagellin molecules

I suggest that the flagellin molecules from different strains of *Salmonella* all have nearly the same shape. The observed forms of the filaments shown in Fig. 1 and elsewhere^{8,9,12,19,20}, can all be understood in terms of small changes in configuration of the unit. These might occur in a given strain in response to a change in the pH (compare ref. 7) or as differences between the flagellin molecules of different strains resulting from changes in a few residues on the polypeptide chain.

I have described the essential features of the model; but various other features are not essential. For example, the four outer bonding sites need not form a plane figure; an arbitrary tetragon has essentially the same packing properties. Also, the alternative configurations of the unit need not involve a change

within the unit; it is also possible to make the model work by having a bi-stable bonding site at one of the corners of the unit. Anyhow, the design features of the subunit are consistent with what is known about the chemistry and morphology of protein molecules. More details of the three-dimensional geometry of packing, and a closer examination of experimental data^{9,12,19,20} will be presented elsewhere.

Dr A. Klug suggested this problem to me, and I thank him for sustained help. I thank Dr S. Asakura and Dr T. Iino for sending advance reports, and Messrs A. A. Barker, E. J. W. Taylor and R. Denston for help in making physical models.

Received February 4; accepted March 26, 1975.

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letters to nature

Significance of the angular diameter-redshift relation

HEWISH, Readhead and Duffett-Smith¹ have reported that interplanetary scintillation techniques reveal an absence of small-diameter sources at the largest redshifts. They have discussed the cosmological implications of the result using standard, homogeneous Friedmann models of the Universe. Here I point out the important effects on the angular diameter-redshift relation caused by small scale inhomogeneities.

The observational relations in the standard, homogeneous Friedmann models (HFM) have been generalised by Dyer and Roeder² to take into account the fact that the Universe may be quite inhomogeneous on the scale of galaxies and clusters of galaxies. The inhomogeneities are put into the models following the method of Schücking³ and Kantowski⁴. In these inhomogeneous Friedmann models (IFM) the total density may be written

$$\rho = \rho_1 + \alpha\rho \quad (1)$$

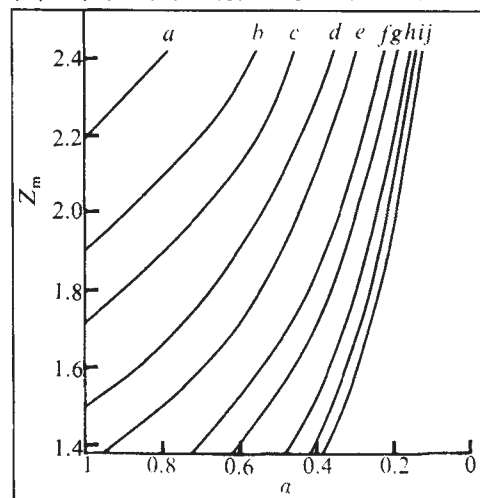
where ρ_1 denotes the density of inhomogeneities, and α , the homogeneity factor, lies in the range $0 \leq \alpha \leq 1$. If $\alpha = 0$, the model is completely inhomogeneous; if $\alpha = 1$, $\rho_1 = 0$ and the model is an HFM. If the lines of sight to distant sources do not pass too close to intervening galaxies, so that shearing of the light beam can be neglected, the distance-redshift relations for the IFM can be expressed as a simple hypergeometric differential equation which gives the solid angle distance, D_s , as a function of the acceleration factor, q_0 , the

redshift of the source, z , and α . Since the projected area of a source, A , and the solid angle it subtends Ω , are related by

$$A = D_s^2 \Omega \quad (2)$$

it is clear that minimum angular diameter occurs at the redshift for which D_s is a maximum.

Fig. 1 The redshift of minimum angular diameter, Z_m , plotted as a function of α . The various Friedmann models specified by letters in the diagram have the following values of q_0 : a, 0.1; b, 0.15; c, 0.2; d, 0.3; e, 0.4; f, 0.6; g, 0.8; h, 1.2; i, 1.6; j, 2.0.



The redshifts of minimum angular diameter, Z_m , have been calculated as a function of q_0 and α in the zero-shear IFM (Fig. 1). The determination of Z_m is, in itself, not sufficient to determine q_0 . Thus, for example, if $Z_m = 1.6$, then $0.25 \leq q_0 \leq 2.0$ corresponds to $1 \geq \alpha \geq 0.3$; and to determine q_0 requires that α be specified. Note that if $\alpha = 0$ there will be no minimum angular diameter regardless of the value of q_0 .

The interpretation of further observations of the angular diameter-redshift relation may provide a simple test for the existence of an intergalactic medium, if the acceleration factor can be estimated independently.

This work has been supported by the National Research Council of Canada.

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Received February 13; accepted April 1, 1975.

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Harmonic analysis of the light of DQ Herculis

SEVERAL classes of interacting binary stars, such as the dwarf novae, nova-like variable stars and X-ray stars, present many examples of rapid periodic optical variations. DQ Herculis (nova 1934) is, however, the only nova known to show such variations. They are of low amplitude, generally less than 0.04 mag semi-amplitude, and have a period of 71.065459 s (refs 1–3). We report here a study of the harmonic components of the 71-s periodicity, and show that the results place severe limits on the available models of the variation.

The observations were made in June, August and September, 1973 at the Cassegrain focus of the 2.08-m telescope of the McDonald Observatory using the high speed two-channel photometer developed by Nather and Warner⁴. The data consist of six observing runs totalling 14.4 h of photometry of DQ Herculis; data taken during eclipse were not included. Individual power spectra were computed for each of the observations and these spectra were then averaged together to produce the power spectrum shown in Fig. 1. The frequencies corresponding to the 142-s subharmonic and to the higher harmonics of the 71-s variation are indicated.

The prominent peak corresponds to the 71-s periodicity and is nearly three orders of magnitude above the noise. At the subharmonic frequency, the contribution to the power spectrum is consistent with the surrounding noise level, which is dominated by the optical flickering of DQ Her. The power at the subharmonic frequency is less than the power at the fundamental frequency by a factor of 310 (17.6 in amplitude).

There are no highly significant harmonic components to the 71-s variation. There is, however, evidence for the existence of the first harmonic, since the highest point of the 1,680 points in the power spectrum that lie at frequencies higher than 0.016 Hz is found precisely at the frequency of the first harmonic. After subtraction of noise, it is found that the contribution of this component is less than that of the fundamental by a factor of 600 in power (24 in amplitude). These values correspond to a maximum semi-amplitude for the first harmonic of 0.0017 mag. The remainder of the observed power spectrum has a shape and level consistent with white noise. A conservative upper limit

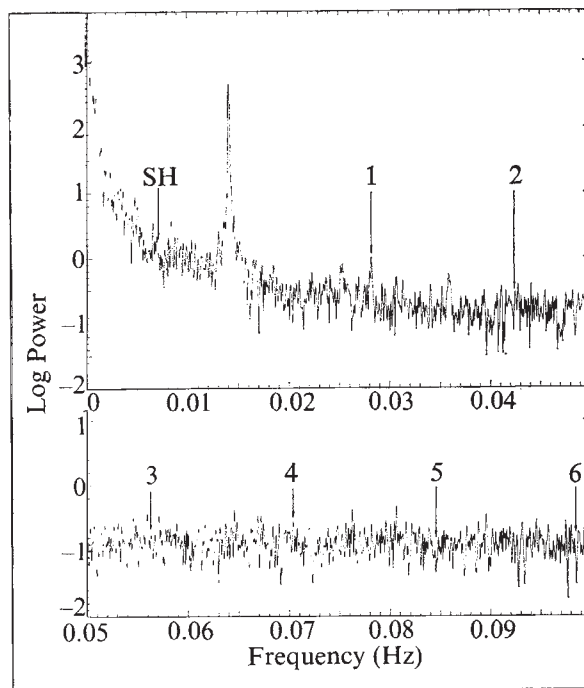


Fig. 1 Log of averaged power of the light of DQ Her showing the harmonic character of the 71-s periodicity. Frequencies corresponding to the 142-s subharmonic (SH) and to the higher harmonics (numbered) are indicated by arrows.

to periodic components in the frequency range of 0.028–0.1 Hz is 0.0014 mag.

Two major groups of models have been advanced to explain the 71-s variation of DQ Her: non-radial white dwarf pulsations and a rotating white dwarf with a dipole magnetic field^{5–8}. In general, a rotating white dwarf with a dipole magnetic field should produce a 142-s subharmonic because of physical and geometric asymmetries between the two poles. On the other hand, pulsational models could also require significant contributions to the power at the harmonic frequencies. Our study has shown that the 71-s variation of DQ Her is a sinusoid of remarkable purity, with a small harmonic contribution to the power occurring only at the frequency of the first harmonic. Consequently, no model of the variation can be considered complete unless it is consistent with this purity.

We thank Mr John McGraw for assistance and discussion, and Dr E. L. Robinson for comments and suggestions.

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Received February 24; accepted April 1, 1975.

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Short-wavelength magnetic anomalies in a region of rapid seafloor spreading

THE main features of the geomagnetic time scale for 0–3 Myr BP are well defined^{1–3}, but the existence and/or number of short events (polarity intervals lasting less than 50,000 yr) is still under discussion. Interest in correlating these short events with features in marine magnetic profiles has focused on three short-wavelength anomalies: anomaly X^{4,5} which appears at approximately 2.3 Myr BP; anomaly W^{6–7} at 2.0 Myr BP; and a short-wavelength feature in the centre of the central anomaly^{3,6,8,9}. These anomalies appear rarely, and then usually only in profiles from regions of rapid spreading. We present here an analysis of a remarkable new set of data from just such a region. Our results provide evidence of the existence of two, or possibly three, short events in the interval 0–3 Myr BP, and of the existence of a magnetised ridge at the precise centre of seafloor spreading.

Our data consist of 14 profiles from a survey of the East Pacific Rise (EPR) crest, centred at 20°S (Fig. 1), where the total spreading rate is about 161 mm yr⁻¹. This survey was completed in the spring of 1973 by the NOAA ship Oceanographer. Navigation was entirely by satellite. Total-field intensity data collected during this survey were reduced to anomaly form¹⁰, and data collected in the vicinity of seamounts or of obvious structural disturbances in the seafloor were discarded. The remaining profiles were projected on to a great circle parallel to the spreading direction—103°—and redigitised to a constant sample interval. These profiles were then transformed to the pole using the method of Blakely and Cox⁷, and adjusted to a uniform spreading rate between known³ magnetic events: the Brunhes–Matuyama reversal boundary at 0.7 Myr BP, the Jaramillo Event centred at 0.92 Myr BP, the Olduvai Event centred at 1.73 Myr BP, and the Matuyama–Gauss reversal boundary at 2.41 Myr BP. Stacking the resulting profiles reduces the random noise in the data set and enhances coherent magnetic anomalies^{11,12}. Signals other than those from magnetic reversals, such as from linear

Fig. 1 Trackline map of the 20°S area over the East Pacific Rise. Inset, location of survey area.

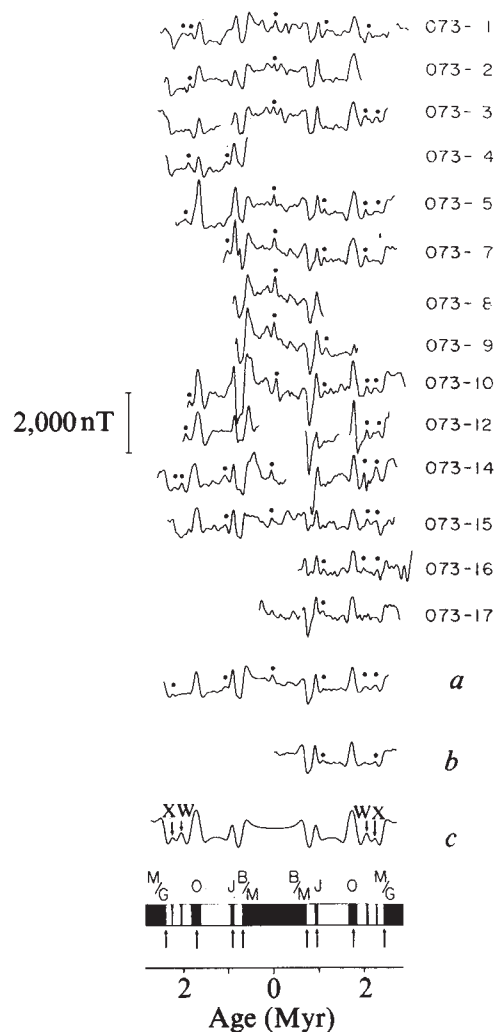
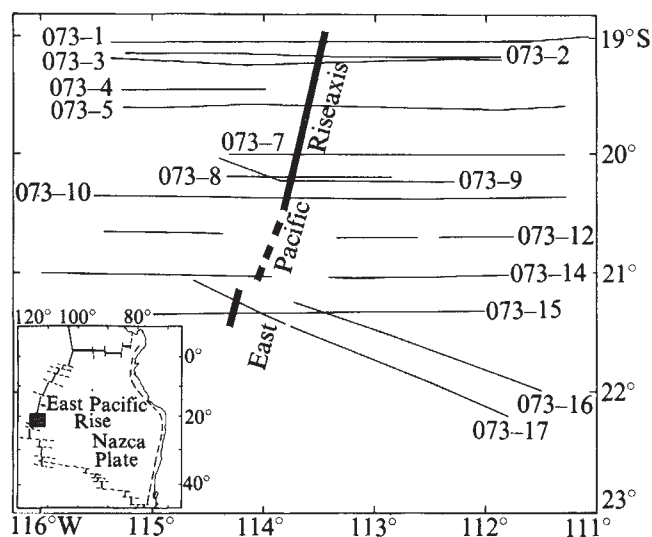


Fig. 2 Individual magnetic anomaly profiles from tracklines shown on Fig. 1. The profiles have been transformed to the pole and adjusted to a uniform spreading rate. *a*, Average of all 14 profiles; *b*, average of profiles after folding in middle; dots, short-wavelength anomalies of controversial origin discussed in text. *c*, Model; arrows, points of 'known' age³; black, normal polarity. B/M and M/G Brunhes–Matuyama and Matuyama–Gauss reversal boundaries, respectively; J and O, Jaramillo and Olduvai events, respectively. The model profile (*c*) was calculated from a crustal layer with top and bottom at 3.0 and 3.5 km respectively. Two brief normal polarity events are centred at 2.05 (W) and 2.25 (X) Myr BP.

topographic features parallel to the rise axis or from intensity fluctuations of the palaeofield¹³, would also be enhanced.

At 20°S on the EPR, the contribution to the magnetic profile from the abyssal-hill topography is minimal except in the one case discussed here. We have no way, however, of differentiating between anomalies produced by significant palaeointensity fluctuations and anomalies produced by true, short events. For the sake of simplicity we will assume that the short-wavelength anomalies discussed here are the result of brief reversals.

Figure 2 shows the individual profiles and the resulting averages. The computer-generated profile (Fig. 2c) was obtained by assuming a horizontal crustal layer with its top and bottom at 3.0 and 3.5 km, respectively. To approximate the imperfection of the crustal recording of geomagnetic reversals, the magnetisation of the layer was

convolved with a normal distribution^{14,15} thus producing a smooth transition in the polarity of the magnetisation at any reversal boundary. A standard deviation of 4 km for the normal distribution was determined by repeated trials. The time scale used to generate the model is shown at the bottom of Fig. 2. This scale is essentially from Klitgord³ except that two additional short events are included: the first lasting from 2.03 to 2.06 Myr BP and the second from 2.24 to 2.26 Myr BP.

The anomaly occurring on 83% of our profiles at about 2 Myr BP (Fig. 2) has been reported in other studies of marine magnetic profiles⁵⁻⁷ and was labelled 'W' by Emilia and Heinrichs⁵. This anomaly probably correlates with the Reunion events, but a controversy exists about the number, age, and duration of these events. A study of volcanic rocks on Reunion Island revealed only one normal polarity event¹⁶, although other evidence suggests that there may be two^{1,17} or even three¹⁸ normal polarity events clustered near 2 Myr BP. Some of this other evidence is, however, based in part on an assumed correlation between an apparently bimodal distribution of age polarity data within the span of about 1.95–2.15 Myr BP and the two small marine magnetic anomalies W and X^{1,17}.

McDougall and Watkins¹⁶ point out that all these dates, because of their nearly overlapping errors, may correspond to the one normal event that they found at Reunion Island and which they dated at 2.02 ± 0.02 Myr BP. They also suggest that this event corresponds to anomaly W, but that anomaly X, dated at 2.3 ± 0.1 Myr BP⁵, is significantly older than the event found at Reunion Island¹⁶. We concur with this suggestion. Unfortunately, because of the large transition width at the reversal boundary and because of inherent limitations on the resolution possible with marine data^{11,12}, we cannot determine whether anomaly W was produced by more than one event. We can only conclude that it is adequately modelled by a single short event lasting from 2.03 to 2.06 Myr BP.

The anomaly located at about 2.3 Myr BP in Fig. 2 correlates with anomaly X reported in previous marine studies⁴⁻⁷ and possibly with a short event found in sediments from south-western Ethiopia¹⁸. Anomaly X appears on 56% of the profiles shown in Fig. 2 and is better represented on the eastern flank of the EPR than on the western flank. We conclude that this anomaly is adequately modelled by a short event lasting from 2.24 to 2.26 Myr BP.

The existence of a brief, normal polarity event about 1.07 Myr ago has been indicated in studies of sediment cores^{19,20} and volcanic rocks^{21,22} but to our knowledge has not been reported with any certainty^{3,6} from marine magnetic profiles. A small positive anomaly that would be about 1.1 Myr old appears on 52% of our profiles but is not strikingly evident in the average profiles. Our unpublished data suggest variations in the spreading regime here during Matuyama time and these variations could cause slight offsets among the profiles. Such small-scale variations in spreading rates would preclude precise stacking of this short wavelength anomaly, as with the two anomalies already discussed. The identification of this anomaly as a short event occurring 1.1 Myr ago must, therefore, remain tentative.

The spreading axis of the EPR is commonly denoted by an axial block or peak several kilometres wide and a few hundred metres high²³. In the 20°S survey area there is a small positive magnetic anomaly of 50–100 nT associated with the axial peak found on nearly all crossings of the axis (91% of the profiles in Fig. 2). This anomaly is best displayed on lines 5–9 of Fig. 2 and shows up on the average profile. This small anomaly has been recognised in various areas by other authors and two causes have been proposed for it. Emilia and Heinrichs⁶ proposed that the Blake reversed polarity event, dated at 0.108–0.114 Myr BP $\pm 10\%$ (ref. 8) caused flanking magnetic lows resulting in a

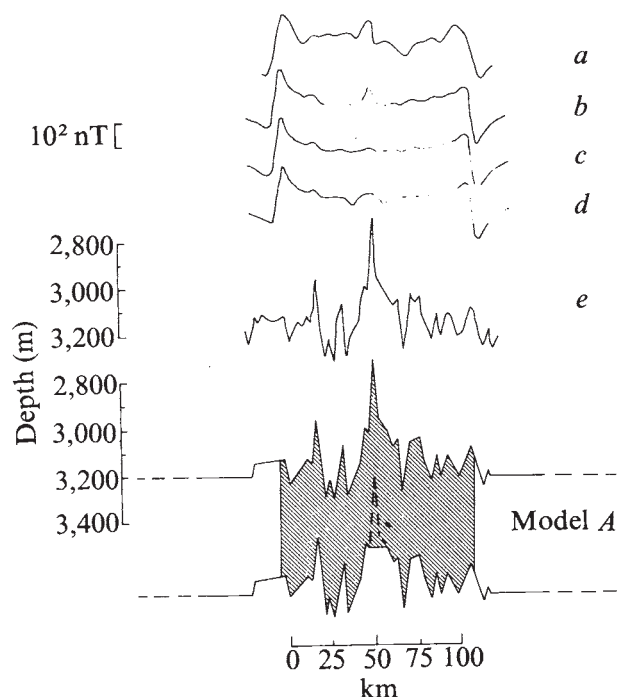


Fig. 3 Observed (a) and calculated (b–d) magnetic anomalies along line 073–5; b, assuming central peak atop a magnetised layer 3.0–3.5 km deep; c, assuming base of layer faithfully follows the surface; d, assuming structure of c and including Blake Event; e, observed bathymetry. Remanent magnetisation is $0.015 \text{ e.m.u. cm}^{-3}$; shaded regions, normal polarity.

relative, central magnetic high. Sclater and Klitgord⁹ and Klitgord³ found a short-wavelength magnetic anomaly associated with the axial peak of the Galapagos spreading centre and related it to an excess of magnetised material within the axial peak.

In an attempt to discern which of these causes may be correct, we constructed several magnetic models of the central anomaly of survey line 5. The models all incorporate a magnetised layer 0.5 km thick that conforms to the observed bathymetry and has a uniform intensity of remanent magnetisation of $0.015 \text{ e.m.u. cm}^{-3}$ (Fig. 3). In model A we have followed the suggestion of Sclater and Klitgord⁹ and assumed that the central peak of the EPR rests on top of usual 0.5-km-thick basaltic basement—assumed in this case, to lie at a depth between 3.0 and 3.5 km (Fig. 3). The resulting profile (Fig. 3b) provides the best match of all the models which we tested, approximating the short-wavelength anomaly in both amplitude and waveform reasonably well. The second model is similar to the first, except that the central peak is considered to result entirely from block faulting so that the layer is 0.5 km thick throughout. The second profile displays essentially no magnetic anomaly over the central topographic peak (Fig. 3c). The third model (Fig. 3d) is the same as the second but contains the Blake reversed polarity event; the profile calculated from this model has small negative anomalies flanking the central portion, but the overall waveform of the central anomaly is noticeably different from the observed data (Fig. 3d).

The close match between the calculated profile (Fig. 3b) and the observed profile (Fig. 3a) suggests that the short-wavelength anomaly associated with the axial peak is the result of a pile of magnetised, extrusive basalt resting on top of a layer of uniform thickness and supports the initial presentation of this idea⁹. Furthermore, our data indicate that the central peak need not be more intensely

magnetised than the adjacent material to produce the observed anomaly. As is the case with all modelling efforts, however, we could construct other more complicated models with some combination of the first and third models (Fig. 3b and d) that would also result in a calculated profile similar to those observed.

In summary, magnetic anomaly data from the EPR at 20°S, where the total crustal accretion rate is 161 mm yr⁻¹ reveal several short-wavelength magnetic anomalies. The oldest of these is about 2.25 Myr old and is correlated with the previously reported anomaly X^{4,5}. A second small anomaly approximately 2.05 Myr old may be the result of one or two closely spaced Reunion events and is correlated with marine magnetic anomaly W⁵. A brief period of normal polarity about 1.1 Myr ago may be reflected by some individual profiles (Fig. 2) but does not show up well in the average profile. Finally, a short-wavelength anomaly always found in conjunction with the topographic axis of the EPR apparently results from an excess of uniformly magnetised material at the rise axis.

We thank B. H. Erickson who was responsible for the collection and initial processing of the data and Allan Cox for reviewing the manuscript. This work was supported by the US National Science Foundation through the IDOE Nazca Plate Project.

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Received February 10; accepted March 2, 1975.

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Plate tectonic and palaeomagnetic implications for the age of the Deccan Traps and the magnetic anomaly time scale

If reconstructions of plate tectonic movement based on magnetic anomaly data are found to be inconsistent with reconstructions based on palaeomagnetic data (see refs 1-3) it may indicate that one or more of the basic assumptions of the plate tectonic model is erroneous; the age or identification of a particular anomaly may be incorrect, the pole positions or sample ages may be unreliable, or there may be an error in assessing plate rigidity. We present an example of inconsistency between the two types of data and explore its implications for the chronology of magnetic reversals during the late Cretaceous and early Tertiary and for the age of the Deccan Traps.

We have previously⁴ reconstructed the major plates in the Atlantic and north-eastern Indian Ocean at the time of anomaly 24 (approximately 60 Myr ago according to

the magnetic anomaly time scale of Heintzler et al.⁵). Anomaly 24 is particularly well defined in these oceans, and we estimated that the uncertainty of the relative positions of the plates is about 200 km. Yet when palaeomagnetic poles for Europe and India, implicitly assumed also to be 60 Myr old, are reconstructed according to the positions of anomaly 24 in the Atlantic and Indian Oceans, the pole positions fall short of one another by more than 2,000 km (Fig. 1). It seems reasonable to assume that the plates are rigid, as the only obvious internal deformation has occurred in the East African Rift System and is clearly negligible compared with the discrepancy obtained. Possibly the best sets of palaeomagnetic data are from the North Atlantic Tertiary Volcanic Province and the Deccan Traps, (Table 1, Fig. 1)^{6,7}. Thus, the age of either the North Atlantic Tertiary Volcanic Province or the Deccan Traps, or anomaly 24, must be significantly different from 60 Myr.

The dating of the North Atlantic Tertiary Volcanic Province has been particularly thorough. Nearly all of the measured potassium-argon and rubidium-strontium determinations have given ages of 50-65 Myr (Table 1).

The age of the Deccan Traps has been less well determined. Although measured ages range from 40-65 Myr (refs 8-10), argon loss seems to have occurred in some of the samples, and the Deccan Traps could have been extruded entirely between 60 and 65 Myr ago^{8,9,10}. Thus, although the few radiometric ages that exist for the Deccan Traps may indicate that both the Traps and the North Atlantic Tertiary Igneous Province were erupted simultaneously the Deccan Traps may, in fact, be a few million years older. At the same time, recent revisions of the magnetic time scale assign to anomaly 24 an age of 56 Myr (ref. 11) or 53 Myr (R. L. Larson, personal communication). Thus, uncertainties in the ages of both the Deccan Traps and anomaly 24 allow some flexibility in reconciling differences of calculated pole positions at the time of anomaly 24. In fact, because of the especially rapid motion of India with respect to both the pole¹² and the surrounding plates¹³ during the late Cretaceous and early Tertiary, the ages of both the Deccan Traps and anomaly 24 are critical; an older age for the Deccan Traps or a younger

Fig. 1 Equal area projection of present palaeomagnetic pole positions from the North Atlantic Province (crosses); present Deccan Trap pole positions (open circles); reconstructed pole position of Deccan Traps (in their positions relative to Europe at the time of anomalies 24 and 32, respectively), (closed and dotted circles).

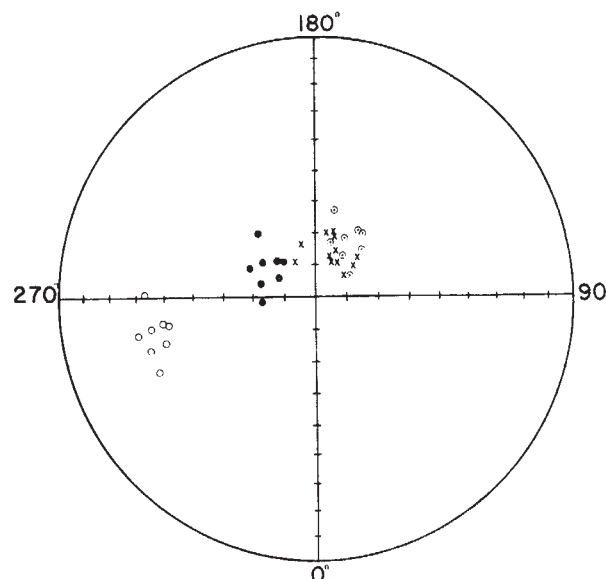


Table 1 Selected palaeomagnetic poles and ages from the North Atlantic Tertiary Volcanic Province and the Deccan Traps*

Rock unit	Age (Myr)	Ref.	Pole		Ref.
			Latitude (°N)	Longitude (°E)	
North Atlantic Tertiary					
Volcanic Province					
Faeros Island volcanics	53–59	14	77	161	15
Skye lavas and intrusives	54 ± 3	16	74	157	17
Ardnamurchan gabbro	55 ± 6	18	69	165	17
	60 ± 3	19, 20			
Rhum gabbro			69	171	17
Mull intrusives	60 ± 10	18	72	133	21, 22
Arran dykes	55–65	23	78	149	24
Antrim igneous suite	49.9 ± 2.2	25	78	145	26
Lundy dykes	52 ± 2	27, 28	75	130	29
Ayrshire dykes			79	128	30
Tertiary dykes, Scotland	34–57	31	73	197	31
Tertiary dykes, UK			77	211	32
St Kilda	56–64	33			
Northern Ireland igneous rocks			70	163	34
Deccan Traps					
Deccan Traps (McElhinny's average)			30	281	6, 12
Amarkantak lavas			33	294	35
Mount Pavargash lavas	59–63	9	39	286	36
	66	10			
Aurangabad lavas			33	287	37
Deccan Traps, western Ghats			35	280	38
Mount Gima lavas and intrusives	65	10	41	280	39
Mahabaleshwar lavas	≥ 61.5	10			
Malwa Plateau lavas			33	269	40
Jalna lavas			39	279	41

* McElhinny⁴ and Bott⁴² were used as guides for selecting and combining these data.

age of anomaly 24 will reduce the discrepancy in reconstructed pole positions.

To explore this further we reconstructed the positions of the lithospheric plates with respect to Europe at the time of anomaly 32, with assigned ages of about 75 Myr (ref. 5) and 70.5 Myr (ref. 11). The average pole positions again disagreed, by about 600 km, but in the opposite sense from the disagreement obtained using anomaly 24. The age of the Deccan Traps is apparently less than that of anomaly 32.

Because Europe moved relatively little with respect to the pole during the Tertiary and Cretaceous⁶, the North Atlantic Tertiary Province probably moved very little with respect to the pole during the period when anomalies 24 to 32 formed. Assuming this, we can use a reconstruction that brings the two sets of palaeomagnetic poles into approximate coincidence to constrain the age of the Deccan Traps or the anomalies. Assuming an approximately constant rate of motion between India and Europe between the times of anomalies 24 and 32, the age of the Deccan Traps can be estimated for various proposed ages of anomalies 24 and 32. Similarly, using given ages of the Deccan Traps and one of these anomalies, the age of the other anomaly can be estimated. Table 2 summarises several combinations of assumed and calculated ages.

Using the time scale of Sclater *et al.*¹¹, the Deccan Traps are calculated to be only 68 Myr old (line *b*, Table 2). If we accept the age found by Sclater *et al.*¹¹ for anomaly 32 but Larson's age determination for anomaly 24 (personal communication), then the calculated age for the Deccan Traps is 67 Myr (line *c*, Table 2). Given the uncertainties in all of the ages, reconstructions, and palaeomagnetic poles, 67 or 68 Myr is probably indistinguishable from the measured ages of 60–65 Myr^{9–10}.

Similarly, assuming that the Deccan Traps are 65 Myr old, then anomaly 32 becomes younger than 70 Myr old (lines *d* and *e*, Table 2). A small change in the assumed age of the Deccan Traps produces a large effect on the age of anomaly 24 (compare lines *b* and *c* with line *g* in Table 2). Because of the uncertainties in the dating of rocks, an accurate determination of the age of anomaly 24 by this

method will be difficult. Nevertheless, anomaly 24 must be younger than 60 Myr old.

Table 2 Assumed and estimated ages (Myr) of anomalies 24 and 32 and of the Deccan Traps

	Deccan Traps	Anomaly 24	Anomaly 32
<i>a</i>	72	60 (5)*	75 (5)
<i>b</i>	68	56 (11)	70.5 (11)
<i>c</i>	67	53†	70.5 (11)
<i>d</i>	65 (9)	53†	68
<i>e</i>	65 (9)	56 (11)	66
<i>f</i>	65 (9)	25	75 (5)
<i>g</i>	65 (9)	45	70.5 (11)

* Number in parentheses gives reference; ages not referenced are estimated.

† R. L. Larson, personal communication.

Thus, reconstructions of the Indian and Eurasian plates, using marine magnetic anomalies and differences in the position of palaeomagnetic poles obtained from these plates together place constraints on the ages of both the anomalies and the samples studied palaeomagnetically. These results are consistent both with results of Sclater *et al.*¹¹ and Larson (personal communication), suggesting that anomaly 24 is younger than suggested by Heirtzler *et al.*⁵, and with the idea that the Deccan Traps may be older than measured. The results presented here are also consistent with an age for anomaly 32 younger than that suggested by Heirtzler *et al.*⁵. Plate reconstructions are apparently sufficiently accurate to be used as a laboratory tool for age dating and 'stratigraphic' correlation over large distances.

One of us (J.F.) did this work while on leave from the Centre Océanologique de Bretagne in the Department of Earth and Planetary Sciences of MIT and thanks X. Le Pichon, F. Press, C. Riffaud and J. G. Sclater for providing

this opportunity. This research was supported by the National Science Foundation.

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Mechanism for nucleation and growth of manganese nodules

FERROMANGANESE oxide crusts and concretions cover vast areas of the sea floor^{1,2} and the relatively high concentrations of cobalt, nickel and copper in manganese nodules, together with the high population densities of polymetallic nodules on certain sea beds, such as the North Pacific Equatorial Belt³, mean that these marine sediments are potential ore deposits. A fact that is often overlooked, however, is that in many sedimentary environments most of the manganese and iron does not accumulate in the form of ferromanganese nodules, but instead is distributed throughout the sediment in the form of dispersed microscopic concretions⁴. A fundamental problem in accounting for the origin and distribution of polymetallic nodules is to explain the nucleation and growth of the host ferromanganese oxide phases. We demonstrate here that crystal-structure

relationships between host manganese (IV) oxide and iron (III) oxide hydroxide phases control the authigenesis, mineralogy and metal enrichments of manganese nodules.

Observations of the interiors of manganese nodules under the microscope⁵, together with electron microprobe analyses⁶⁻⁸, reveal the intimate associations and oscillatory intergrowths of the manganese- and iron-bearing minerals. Most nodules have formed about a nucleus consisting of pumice, altered basaltic rock or glass, clays, tuffaceous material, or hard parts of organisms (shark teeth, whale bones, globigerina tests, coral or radiolaria tests). Microprobe analyses of the boundaries between the nuclei and ferromanganese oxide layers^{9,10} reveal that a hydrated iron oxide phase coats the nucleating agent before the cyclic deposition of Mn oxides plus trace metals and Fe oxyhydroxides occurs. The hydrated iron oxide substrate is probably precipitated at interfaces where the pH is locally high as a result of dissolution and hydrolysis of the carbonate, silicate and phosphate anions derived from the nucleating agent¹⁰. Such evidence for the growth of the Mn oxide phase on a hydrated Fe oxide substrate, together with the intimate association of the ferromanganese oxides across manganese nodules, suggests that crystallographic relationships exist between the constituent Mn and Fe oxide minerals.

Table 1 lists selected manganese (IV) oxide and iron (III) oxide hydroxide minerals for which crystal structure data are available. Todorokite ("10 Å manganite"), the crystal structure of which has not been determined, is also an important constituent of manganese nodules. The acicular habits of todorokite crystallites in terrestrial deposits¹¹ and in manganese nodules¹² suggest to us that todorokite may have a tunnel structure similar to that of hollandite or psilomelane. Several of the minerals listed in Table 1 are isostructural; thus, each of the pairs goethite and ramsdellite, akaganéite and the hollandite group, and probably FeOOH.xH₂O and δ-MnO₂, have identical crystal structures or contain similar planes of atoms. In such isostructural minerals, oriented intergrowths or epitaxy of one crystalline phase on the other may occur, provided that dissimilarities of lattice parameters or interplanar spacings are not large. Indeed, epitaxial growths of ramsdellite ($a_0 = 4.53$ Å, $b_0 = 9.27$ Å, $c_0 = 2.87$ Å) on goethite ($a_0 = 4.65$ Å, $b_0 = 10.0$ Å, $c_0 = 3.04$ Å) are observed in geodes^{13,14}. The mismatch of unit cell dimensions between these two minerals is less than 7%, and seems to be small enough to enable epitaxy between goethite and ramsdellite. The ramsdellite crystal structure¹⁵ contains Mn atoms surrounded by six oxygens at the corners of an octahedron. The [MnO₆] octahedra share edges with adjacent octahedra to form double chains which are cross linked to adjacent double chains by corner sharing of oxygen atoms forming structure with orthorhombic symmetry. In goethite, the double chains of edge-shared [Fe(O,OH)₆] octahedra are further linked by hydrogen bonds¹⁶. Another way of viewing these two structures is in terms of a hexagonal close-packed oxygen framework in which Mn⁴⁺ or Fe³⁺ cations are distributed in an ordered array among the octahedral sites. The structure of hydrated ferric oxyhydroxide polymer is believed to contain Fe³⁺ ions in a hexagonal close-packed network^{17,18}, but the cations are randomly distributed in the octahedral sites with but random order among them. The phase δ-MnO₂ is considered to be a disordered birnessite^{19,20} and to contain fragments of the layers of edge-shared [MnO₆] octahedra with small periodicities of stacking along the *c* axis. Thus, δ-MnO₂ also consists of a network of hexagonal close-packed oxygens containing Mn⁴⁺ ions in the octahedral sites. The structural similarities between δ-MnO₂ and FeOOH.xH₂O make them amenable to epitaxial intergrowths.

We conclude, therefore, that the nucleation and growth of many manganese nodules is initiated by the epitaxial growth of δ-MnO₂ on to the FeOOH.xH₂O substrate coating the nucleating agent. Episodic growths of these two phases on one another lead to the intimate association of Mn and Fe minerals in manganese nodules. Such intergrowths of δ-MnO₂ and FeOOH.xH₂O inhibit the formation of the ordered layer

Table 1 Isostructural manganese and iron oxide minerals

Manganese (IV) oxides	Manganese (III) oxide hydroxides	Iron (III) oxide hydroxides	Structure type
Pyrolusite β -MnO ₂	—	—	Rutile
Ramsdellite MnO ₂	groutite α -MnOOH	*Goethite α -FeOOH	Diaspore
*Nsutite γ -MnO ₂	—	—	Pyrolusite + ramsdellite
Hollandite group (Ba,K,Pb,Na) ₁₋₂ Mn ₈ O ₁₆ ·xH ₂ O	—	*Akaganèite β -FeOOH	Hollandite
*Psilomelane (Ba,K,Mn,Co) ₂ Mn ₅ O ₁₀ ·xH ₂ O	—	—	Psilomelane
—	—	*Lepidocrocite γ -FeOOH	Boehmite
—	—	Un-named δ -FeOOH	Disordered goethite
—	Feitknechtite β -MnOOH	—	Brucite
—	Manganite α -MnOOH	Un-named ϵ -FeOOH	Manganite Birnessite
*Birnessite ("7 Å manganite") (Ca,Na,Mn)Mn ₂ O ₄ ·3H ₂ O	—	—	
* δ -MnO ₂ ("protobirnessite")	—	*FeOOH·xH ₂ O ("ferrihydrite") (hydrated iron (III) oxyhydroxide polymer)	Hexagonal close-packed oxygen
*Todorokite ("10 Å manganite") (Na,Ca,Mn)Mn ₃ O ₇ ·xH ₂ O	—	—	Unknown

*Reported in manganese nodules.

structure of birnessite. We suggest, however, that post-depositional recrystallisation facilitates long range ordering and may produce periodic stacking sequences characteristic of the birnessite and todorokite structures, the todorokite phase being stabilised by high water pressures¹⁰.

Research supported by the National Science Foundation—IDOE Ferromanganese Nodule Program.

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Hydrocarbons associated with lead–zinc ores at Laisvall, Sweden

BITUMEN and a series of normal alkanes (C₁₈ to C₂₇) have been identified in sphalerite from the Laisvall lead–zinc deposit. This is a stratabound lead–zinc ore in Eocambrian sandstone, situated at the eastern border of the Swedish Caledonides. The deposit is an aberrant member of the

Mississippi Valley ore class¹. The main ore minerals are galena and sphalerite, associated with minor amounts of calcite, barite and fluorite. These infill interstices around the sandgrains, often following sedimentary structures². The lead is markedly radiogenic J-type with a ²⁰⁶Pb/²⁰⁴Pb ratio of more than 20 (ref. 3). Fluid inclusion studies on the sphalerite give homogenisation temperatures around 150 °C (ref. 4). Laisvall is the largest lead concentration (original resources of about 60 × 10⁶ t of 4% Pb) in the 1,000 km long lead- and zinc-rich Caledonian border zone, which includes deposits such as Dorotea and Vassbo.

Samples of the ore and enclosing rocks were taken both from drill cores and hand specimens from the mine itself. Crushed rock samples were washed and shaken three times for 24 h with acetone. The samples were further crushed to less than 63 µm with a pestle and mortar cleaned with acetone. Three grammes of the powder in 5 ml toluene were placed in an ultrasonic bath for 30 min, filtered and evaporated to dryness. The residue was dissolved in 0.10 ml ether–acetone (1:1), and 3 µl of the solution was injected into a glass capillary column OV-1, 25 m long, in an LKB 2091 gas chromatograph–mass spectrometer–computer system.

The results are listed in Table 1. Total extractable organic matter was variable up to a maximum of 100 p.p.m. In most cases the gas chromatogram consisted of a single broad peak, representing a complex mixture of high molecular weight isomers, which can be loosely described as bitumen. In two samples a series of alkanes were identified which made up about 10% of the total extractable material (Fig. 1).

The extractable organic matter was concentrated only in those samples containing sphalerite. Samples rich in galena, silica, calcite, fluorite or barite contained low to negligible amounts of extractable material. Furthermore, all sphalerite samples examined were relatively rich in organic matter. We therefore conclude that the organic material is closely associated with the sphalerite and further, that the organic material is partly situated within the abundant³ fluid inclusions in the sphalerite, although attempts to identify the location of the material (for example by ultraviolet microscopy) have not been successful to date.

Our evidence that this organic material is natural and not an artefact is based on several lines of reasoning. In particular: (1) the enclosing sandstone host rock associated

Table 1 Estimated contents of bitumen and normal alkanes from Laisvall specimens

Specimen no.	Composition	Source	Type	Organic material	
				Estimated total concentration (p.p.m.)	Concentration of normal alkanes (p.p.m.)
L01X	Sphalerite*, calcite, quartz	Nadok orebody	Bitumen	100	Not determined
L02X	Galena*, calcite, quartz	Strand orebody	Bitumen	1	Not determined
L03X	Calcite*, galena, quartz	Lower sandstone (core)	Bitumen	Not detected	Not detected
L04X	Galena*, quartz	Kautsky orebody	Bitumen	1	Not determined
L05X	Galena*, quartz	Nadok orebody	Bitumen	1	Not determined
L06X	Sphalerite*, calcite, quartz	Nadok orebody	Bitumen	100	Not determined
L07X	Barite*, quartz	Lower sandstone (core)	Bitumen	1	Not determined
L08X	Fluorite*, quartz	Lower sandstone (core)	Bitumen	1	Not determined
L09X	Sphalerite*, calcite, silica	Nadok orebody	Bitumen + C ₁₈ -C ₂₇	~40	4
L11X	Sphalerite*, calcite, silica	Nadok orebody	Normal alkanes		
			Bitumen + C ₁₈ -C ₂₇	~40	4

The estimations were made by comparison with a C₁₈ standard. For this reason the estimates of total organic material (as bitumen) are presented as orders of magnitudes, whereas the alkane concentrations are accurate to within ± 1 p.p.m.

*Principal constituent.

with these samples, is essentially impermeable with a porosity approaching zero (M.W., unpublished); (2) similar samples, without sphalerite, contain low, possibly background, amounts of organic compounds; (3) both core and hand specimen samples were examined; (4) analyses of possible contaminating substances give either no measurable organic contamination (such as acetone) or different gas chromatograms (such as machine oil and grease); (5) the laboratory preparation was such that the samples could only be contaminated during the final crushing (that is, after acetone-washing of the preliminary crushed samples and before the ultrasonic extraction) and this stage was rigorously controlled; (6) although the possibility of airborne contaminating material dropping into the samples cannot be entirely precluded, the observations that only samples rich in sphalerite suffered this fate and even in these samples

different gas chromatograms were obtained, seem to rule out even this possibility.

The identified alkanes in specimens L09X and L11X include the high molecular weight paraffins C₁₈ to C₂₇ (Fig. 1). A peak observed after C₂₇ is consistent with squalene. Work is continuing on this material to identify the origin and authenticity of this observation. The carbon preference index (CPI) is very low, about 0.64. The absence of lower molecular weight alkanes and the low CPI with its even number preference indicates that these substances are not derived directly from biological materials. They must have developed as a result of secondary reactions from the original organic substances. This is not only further evidence against contamination of the samples by modern biological substances, but is also consistent with the age and probable history of the deposit. The sphalerite was formed before the

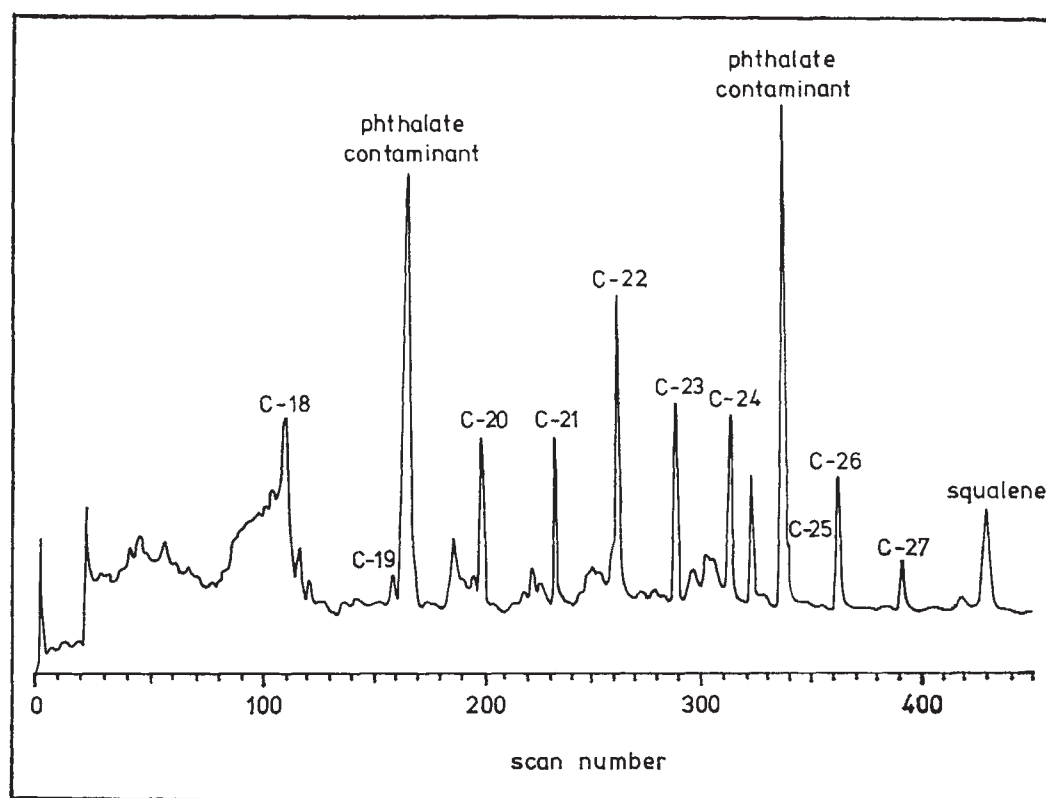


Fig. 1 Gas chromatogram of specimen L09X (sphalerite, calcite and quartz), showing a series of normal alkanes C₁₈-C₂₇ and squalene. Glass capillary column OV-1, 25 m long, from 120 °C to 250 °C at 10 °C min⁻¹.

Caledonian orogeny and during or after the Eocambrian sedimentation, a period of about 200 Myr commencing about 600 Myr BP.

It has been proposed on the basis of isotope studies, that the ore forming fluids involved in the formation of Mississippi Valley-type ores were highly saline oilfield brines¹. The evidence from our study adds further weight to this hypothesis since, in the case of Laisvall, it seems that the solutions contained petroleum-type compounds.

This work was supported by the Swedish Natural Science Research Council and the Boliden Company.

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Received March 17; accepted April 1, 1975.

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Experimental alteration of chlorites into vermiculites by chemical oxidation

VERMICULITE and chlorite minerals occur commonly in soils where they may be an important source of magnesium and micronutrients. Vermiculite adsorbs and retains potassium and ammonium preferentially, which makes it particularly important in exchange reactions involving these cations. Like micas, chlorites in soils may weather to vermiculites^{1,2}. Micas are readily altered to vermiculites in the laboratory by the extraction of potassium. It has not, however, been possible to alter unoxidised, true chlorites to vermiculites by extracting the hydroxide sheets using chemical techniques^{3,4} in the laboratory.

I demonstrate here that pure, unoxidised chlorites (IIB polytype) containing an appreciable amount of ferrous iron may be changed to vermiculites in the laboratory using a simple and effective chemical oxidation technique. I show the importance of the oxidation of structural ferrous iron in this alteration process and describe some properties of the vermiculite products.

The alteration technique consists of reacting chlorites in saturated bromine water on a steambath. Samples of 300 mg of 2–5 μ m chloite were placed in screw cap, Pyrex vials containing 20 ml of saturated bromine water. The vials were capped tightly and placed in the steambath. After the appropriate time the vials were opened and the bromine boiled off. The samples were separated by centrifugation and then treated twice with sodium dithionite⁵ to remove free iron-hydrous and aluminium-hydrous oxides. Portions of the samples were taken before and after the oxidation and dithionite treatments and decomposed in HF (ref. 6). The silicon content was determined colorimetrically⁸ and Fe, Al and Mg were determined by atomic absorption spectrophotometry. Ferrous iron was also measured⁷. X-ray diffraction patterns were obtained from specimens basally oriented on glass slides using an instrument with Fe-filtered, Co radiation.

Figure 1 shows the transformation of high-iron chlorite — $(\text{Al}_{1.57}\text{Fe}_{0.28}^{3+}\text{Fe}_{0.28}^{2+}\text{Mg}_{0.68})(\text{Si}_{3.32}\text{Al}_{0.68})\text{O}_{10}(\text{OH})_8$ — into vermi-

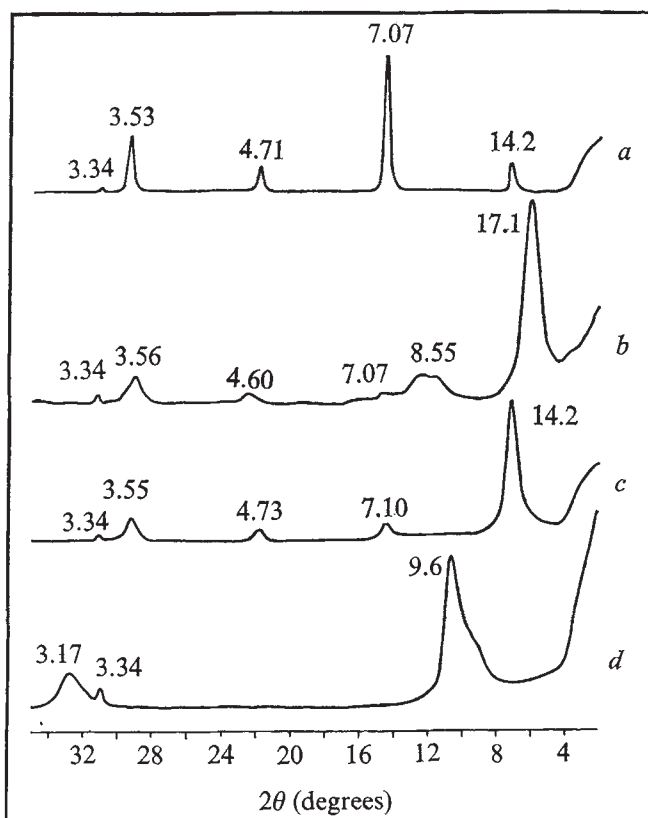


Fig. 1 X-ray diffraction patterns of a high-iron chlorite (diabantite from New Mexico). *a*, Original sample; *b*, *c* and *d*, after 3 weeks' reaction: *b*, saturated with Ca and glycerol; *c*, saturated with Mg and glycerol; *d*, saturated with Ca and heated at 700 °C. Spacings in Å.

culite after three weeks of reaction. The vermiculite product expands to 17.1 Å when saturated with Ca and glycerol (Fig. 1*b*) and to 14.2 Å when saturated with Mg and glycerol (Fig. 1*c*). This expansion behaviour resembles that of low-charge vermiculites with a cation exchange capacity (CEC) of around 130 meq equivalent per 100 g. Heating at 700 °C contracts the layers to 9.6 Å but a shoulder at the low-angle side of this peak indicates some (~20%) remaining chlorite layers. The CEC increased from 1.8 to 100.4 meq per 100 g during the reaction.

A low-iron chlorite — $(\text{Al}_{1.00}\text{Fe}_{0.04}^{3+}\text{Fe}_{0.39}^{2+}\text{Mg}_{1.72})(\text{Si}_{2.62}\text{Al}_{1.38})\text{O}_{10}(\text{OH})_8$ — when treated in the same way as the high-iron chlorite showed very little alteration to vermiculite even after 5 months of reaction (see Fig. 2*a*, *b* and *c*).

The evidence (Figs 1 and 2) suggests that the oxidation of structural ferrous iron is an important mechanism in the alteration of chlorite to vermiculite. This is further brought out by the data in Table 1 which show that the reaction in acid solution without addition of an oxidising agent has little effect on chemical composition and CEC. The X-ray diffraction pattern of this sample also remained identical to that of the original chlorite. In the presence of bromine as an oxidising agent, the composition and CEC change drastically, reflecting the alteration of chlorite to vermiculite. The X-ray diffraction patterns of this sample are shown in Fig. 1*b*, *c* and *d*.

Assuming that the Si-to-Al ratio of the tetrahedral sheets does not change and that 20% of the original chlorite remains unaltered, the formula of the vermiculite portion (80%) in the altered product is $(\text{Al}_{0.44}\text{Fe}_{1.19}^{3+}\text{Fe}_{0.40}^{2+}\text{Mg}_{0.15})(\text{Si}_{3.32}\text{Al}_{0.68})\text{O}_{10}(\text{OH})_2 [\text{Ca}_{0.34}]^{0.68+}$. The number of cations (Al, Fe and Mg) in the octahedral sheet is 2.18 which shows that there was a considerable loss of these cations from the octahedral sheets of the mica-like layers during the alteration process. This was also indicated by a decrease in the

Table 1 Partial chemical analysis of original and altered chlorite (diabantite from New Mexico)*

Cation	Original	Original plus dithionite treatment	Three weeks reaction in HCl (pH 2.0)† followed by dithionite treatment	Three weeks reaction in Br ₂ water (pH 1.8)‡ followed by dithionite treatment
Al	8.93	8.93	9.50	5.97
Fe ²⁺	23.70	25.56	24.10	7.84
Fe ³⁺	2.30	0.44	1.82	9.58
Mg	2.44	2.69	2.62	1.20
CEC (m equivalent per 100 g§)	1.8	6.9	13.1	100.4

*Based on sample weight at 110 °C.

†Initial pH 1.4; final pH 2.0.

‡Initial pH 2.2; final pH 1.8.

§Ca exchanged with Mg.

d(060) spacing from 1.557 Å in the original chlorite to 1.537 Å in the altered product, which comprised approximately 20% chlorite layers. Decreases in the *d*(060) spacings because of losses of octahedral cations have also been reported in the oxidation of biotite⁸.

It has been proposed previously that selective dissolution of the hydroxide sheet is induced by a structural disturbance of this sheet⁹. During chemical oxidation this structural disturbance is probably caused by the oxidation of ferrous iron and its ejection from the hydroxide sheet.

The results reported here indicate that the oxidation and removal of ferrous iron play an important role in the weathering of chlorites to vermiculites in soils. This was also suggested in a study which showed that one extraction by sodium sulphite or sodium dithionite of a partially oxidised iron chlorite resulted in the formation of a regularly interstratified chlorite-vermiculite². The abundance of chelating agents present in most soils would facilitate the removal of iron from the chlorite structure under less acid conditions than those used in my experiment.

As well as providing clarification about chlorite weathering in soils, the ability to change chlorites to vermiculites in

the laboratory provides the opportunity for comparison between the structure and behaviour of chlorite-derived vermiculites and mica-derived vermiculites; it is, however, impossible to do this with soil vermiculites as they are mixed inextricably. Of particular interest in this comparison would be the exchange behaviour with potassium and ammonium, which could well be influenced by the origin of the vermiculites¹⁰.

I thank M. Jaakkimainen and N. M. Miles for their technical help and Drs H. Kodama and J. D. Adshead for reviewing the manuscript.

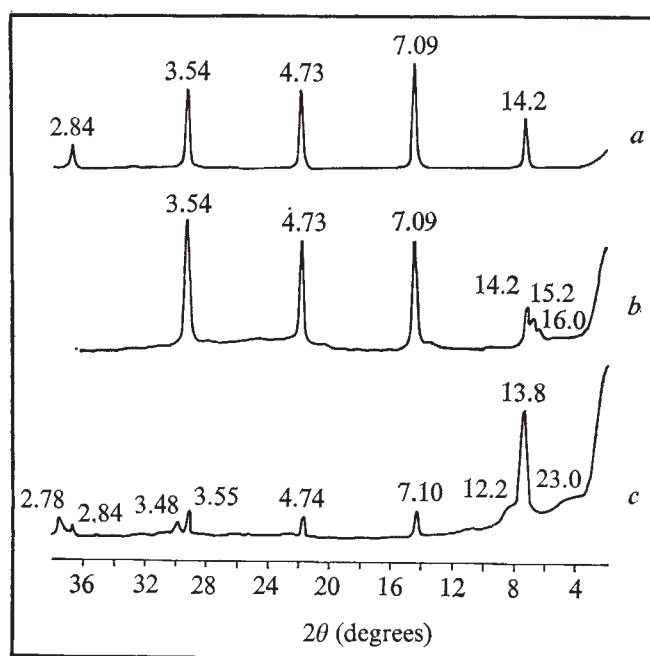
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Received February 7; accepted March 25, 1975.

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Fig. 2 X-ray diffraction patterns of a low-iron chlorite (sherdanite from Brewster, New York). *a*, Original sample; *b* and *c*, after 5 months' reactions at pH 2.0: *b*, saturated with Ca and glycerol; *c*, saturated with Ca and heated at 700 °C. Spacings in Å.



Chemical transport by the Mekong river

MAJOR rivers are important for an understanding of the overall balance of dissolved and particulate matter carried to the ocean^{1,2}. Although Livingstone³ gives a large amount of information on the world surface waters, there is still a lack of data on many important rivers, especially from South-east Asia. These rivers are generally presumed to contribute a great part to the continental and particulate balance^{1,3,4}, but are poorly documented except for the work of Kobayashi in Thailand⁵ and a few analyses performed for the International Association of Scientific Hydrology⁶. The Mekong river is one of the biggest in this region^{7,8} (Table 1). We have previously studied the hydrology of the Tonle Sap-Mekong system⁹ and the influence of the Mekong river on Cambodia Grand Lac water chemistry¹⁰. Here we deal with analyses performed at Phnom Penh (Fig. 1).

At this station the basin area is 663,000 km², 83% of the total drainage area, and mean annual discharge is 14,900 m³ s⁻¹. Between January 1961 and December 1962, 546 daily samples were taken in which temperature, pH and conductivity were measured. The river was at that time affected little by war activities. Once a month a complete water analysis was performed. In this report we set up the mean annual water quality value, compare transport rates

Table 1 Largest rivers of the world according to the water and dissolved salt discharges

River	Water discharge $\text{m}^3 \text{ s}^{-1}$	Basin area 10^3 km^2	Salt discharge 10^6 t yr^{-1}	Reference
Amazon	175,000	6,300	290	14
Congo	39,200	4,000	50	19
Orinoco	30,000	950	50	3
Yang Tse	22,000	1,950	$\approx 100^*$	—
Mekong	21,200	795	60	—
Brahmaputra	19,200	580	≈ 80	—
Mississippi	18,400	3,267	142	15
Parana	18,000	2,800	57	18
Yenissei	17,200	2,600	$\approx 73^\dagger$	1, 13
Lena	16,300	2,430	$\approx 85^\dagger$	1, 13
Ganges	11,610	990	76	—
Danube	6,430	805	60	16
Mackenzie	9,600	1,800	60	17

*Minimum value, on the basis of a $50 \text{ t km}^{-2} \text{ yr}^{-1}$ transport rate.

† Total ionic transport plus 10% for silica transport.

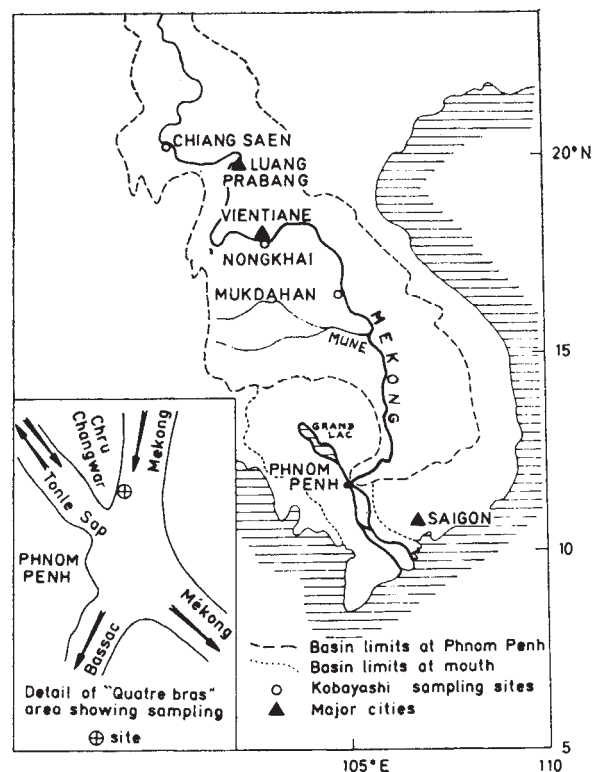
at Phnom Penh with upstream rates in Thailand recomputed from Kobayashi values and compare chemical erosion in the Mekong basin with those of other Himalayan rivers and determine the importance of South-east Asia for the global dissolved balance.

Mean daily total ionic contents (Σ^{+-} in mg l^{-1}) have been computed on the basis of daily conductivities measured at 25°C (measured in $\mu\text{mho cm}^{-1}$) converted into Σ^{+-} according to the relationship

$$\Sigma^{+-} = 0.72 \times \text{conductivity} + 14.3$$

(regression computed for 23 values, $r = 0.97$). The total ionic transport for 1961–62 is $45.5 \times 10^6 \text{ t yr}^{-1}$. Since the water discharge during this period was very close to the mean annual discharges ($14,970 \text{ m}^3 \text{ s}^{-1}$ as against $14,900 \text{ m}^3 \text{ s}^{-1}$), this value can be taken as a fair average. Mean major element contents have been computed on the basis of the 24 instantaneous dissolved discharges corresponding to monthly analyses. Total ionic transport given by this less accurate method is $42.6 \times 10^6 \text{ t yr}^{-1}$ so that annual transport averages for each element were corrected. As dissolved silica is not influencing conductivity, mean SiO_2 transport was not corrected (Table 2). As for the other big rivers¹⁻³, bicarbonate and calcium are the principal ions carried by the Mekong. The proportion of silica (8.5%) is similar to Livingstone's world mean³ (11%).

We have recomputed discharge-weighted concentration

**Fig. 1** Map of area.

averages at three Thailand stations studied by Kobayashi⁵: Chieng Saen, Nongkai and Mukdahan (Fig. 1 and Table 1). We used drainage area values and discharge measurements at Mukdahan¹¹ for estimating monthly discharges at Chieng Saen and Nongkai that were not available to Kobayashi. Transport rates at Chieng Saen are characteristic of the mountainous upper Mekong basin. The total mineral transport rate ($86 \text{ t km}^{-2} \text{ yr}^{-1}$) is relatively low as compared to other high-relief basins such as the Ucayali river in the Andes² ($152 \text{ t km}^{-2} \text{ yr}^{-1}$), or the Rhone river (France) at mouth ($190 \text{ t km}^{-2} \text{ yr}^{-1}$). The transport rates are nearly constant along the 1,850-km river course from Chieng Saen to Phnom Penh. The lower Mekong basin is chiefly exposed to monsoon rains and the relief is much more gentle. The

Table 2 Quality variation along the Mekong river

Location	SiO_2	Ca^{2+}	Mg^{2+}	Na^+	K^+	Cl^-	SO_4^{2-}	HCO_3^-	NO_3^-	Total ionic	Total mineral
Chieng Saen* † 195,000 km^2 1,955 km §	6.9	13.8	2.3	2.9	0.72	1.9	5.8	51.1	0.04	78.5	85.4
Nongkai* † 310,000 km^2 2,650 km	7.1	13.0	2.1	2.5	0.75	1.9	4.3	49.3	0.08	73.9	81.0
Mukdahan* † 390,000 km^2 3,250 km	8.2	13.7	2.1	2.9	0.77	1.9	3.8	51.7	0.11	77.0	85.2
Phnom Penh † 663,000 km^2 3,800 km $^{ }$	6.3	10.1	2.2	2.6	1.4	7.5	2.7	41.2	0.82	68.5	74.8
	8.85	14.2	3.2	3.6	2.0	10.5	3.8	57.9	1.1	96.5	105.1

*Recomputed from data of Kobayashi⁵.

† Transport rate $\text{t km}^{-2} \text{ yr}^{-1}$.

§ Drainage area.

§ Distance from source.

$^{||}$ Concentration in mg l^{-1} .

Table 3 Dissolved transport rate of Mekong compared with other Himalayan rivers

		SiO ₂	Ca ²⁺	Mg ²⁺	Na ⁺	K ⁺	Cl ⁻	SO ₄ ²⁻	HCO ₃ ⁻	NO ₃ ⁻	Total ionic	Total mineral
Mekong	(a)	6.3	10.1	2.2	2.6	1.4	7.5	2.7	41.2	0.82	68.5	74.8
	(b)	5.0	8.0	1.75	2.05	1.1	5.95	2.15	32.8	0.65	54.5	59.5
Ganges	(a)	3.8	9.4	3.0	4.1	1.3	3.2	4.1	48.7	0.5	74.3	78.1
	(b)	3.7	9.15	2.9	4.0	1.3	3.1	4.0	47.5	0.5	72.5	76.2
Brahmaputra	(a)		21.2	3.6	3.1	2.4	5.2		90			
	(b)		12.3	2.1	1.8	1.4	3.0		52			
Mean of Himalayan rivers	(a)	4.9	12.8	2.9	3.4	1.65	5.2	3.5	57.4	0.7	87.6	92.5
World mean	(c)	4.2*	4.9†	1.2‡	1.65‡	0.55‡	2.3‡	4.2‡	16.6‡		31.4	35.6

(a) Transport rate (t km⁻² yr⁻¹)(b) Contribution the Ocean (10⁶ × t yr⁻¹)(c) For the continent surface contributing to the ocean balance, 101 × 10⁶ km², glaciated area excluded.*Recomputed from Livingstone³.†Recomputed from Alekin¹ and Brazhnikova¹³.‡Na + K = 2.15 t km⁻² yr⁻¹ from refs 1 and 13, K ≈ Na/3 according to Livingstone³.

total transport rate in this region can be estimated as 75 t km⁻² yr⁻¹, still two times the world average (see Table 3). Monsoon action over mid-altitude relief is as erosive as cold climate action over high mountains of the upper basin. The order of transported elements is the same from Chieng Saen to Phnom Penh apart from an increase of Cl⁻ between Mukdahan and Phnom Penh that can be explained by high Cl⁻ input between these two stations from Thailand tributaries such as the river Mune³.

The Mekong dissolved transport rates are compared to Ganges and Brahmaputra rates in Table 3. The Ganges value was computed from three single analyses quoted by Raymahashay¹² at Farakka, by Livingstone³ at Calcutta and given by G. K. Seth (unpublished). For the Brahmaputra we found only a single reliable analysis at Pandu¹². So no discharge-weighting has been carried out and these water quality values are inaccurate to the extent of ±50% error for SO₄²⁻ and NO₃⁻. General characteristics for these rivers were taken from the UN-ECAFE commission^{7,8}. The Brahmaputra transport rates are by far the highest. Total transport is up to 145 t km⁻² yr⁻¹, the maximum for any such river, and it can be accounted for by the very high relief of this basin (60% is within the Himalaya range compared with only 25% for the Ganges and the Mekong rivers) and by precipitation that is responsible for a high specific discharge, 36 l s⁻¹ km⁻². Relief is, for Brahmaputra, the main environmental factor that controls dissolved transport; this influence has also been noted for the Amazon basin³ and for rivers in the Soviet Union^{1,13,14}. Ganges transport rates are very similar to the Mekong ones, which may be caused by the similarity of the topographic and climatic features of their basins.

If the transport rates determined at Phnom Penh are extrapolated to the remaining basin area (we assume that there are no major changes arising from rice paddy fields in the Delta), the Mekong carries 1.7% of the global dissolved mineral input to the ocean (3,600 × 10⁶ t yr⁻¹, Table 3). This gives it eighth position in the dissolved input 'league' (Table 1). This classification is somewhat different from that of Gibbs²⁰. The dissolved matter carried by the 10 'top' rivers is around 33% of the total input to the ocean. We have estimated the dissolved transport rate for mid-relief basins exposed to the monsoon as 75 t km⁻² yr⁻¹. If this value is taken as a first estimate for South-east Asian rivers still not investigated in detail (Yang Tse Kiang, Pearl, Irrawaddy, Salween . . .) and for the Indonesia and New Guinea archipelagos rivers, the contribution of South-east Asia from the Ganges to the Yang Tse Kiang to the dissolved discharge to oceans may be more than 30%. It seems necessary, therefore, to study further the geochemical processes in these regions.

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Received February 10; accepted March 25, 1975.

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A fifteen-year record of biotic metabolism in the Northern Hemisphere

THE health and productivity of the biosphere are essential to man's welfare. Several scientists have speculated that global productivity and/or biomass should be increasing because of such factors as plant fertilisation from industrially derived atmospheric CO₂ enhancement^{1,2}. Others have maintained that global primary production and biomass must be decreasing because of pollution effects, such as from acid rain, and forest cutting^{3,4}. There are some (rather controversial) tree-ring data that may support this view^{5,6}. If one or the other of these trends is true, there would be a shift in the absolute and relative rates of global photosynthesis, respiration and their ratio (the PR ratio). Earlier attempts to ascertain any such trends from long term changes in atmospheric CO₂ concentration have been frustrated by difficulties in distinguishing changes in biospheric retention from changes in the uptake or discharge of other reservoirs, principally the sea and industrial sources^{7,8}. We present

here a new model for the examination of such trends that we believe avoids such problems by focusing on potential changes in yearly patterns of atmospheric CO_2 .

A nearly continuous record of the atmospheric concentration of CO_2 has been maintained for the past 16 yr at Mauna Loa, Hawaii (19.5°N, 155.6°W, 3,400 m altitude^{7,9,10}) which is located within the well-mixed tradewind belt. The annual variation in CO_2 content there is 6 p.p.m., roughly representative of the Northern Hemisphere, where the annual variation ranges from about 3 p.p.m. at 10°N to a measured maximum of about 15 p.p.m. at Point Barrow, Alaska (71°N ref. 11). The Mauna Loa data provide the only suitable intensive atmospheric CO_2 record available for the Northern Hemisphere over a sufficient length of time.

These data, which are remarkably smooth and consistent, were obtained with a well calibrated, continuously recording gas analyser, and are corrected for local effects as described by Keeling and coworkers^{9,10}. Data are unavailable for only six of the 178 months of this record, and values for these months are estimated from the least square fit of the data to a function of time which included oscillatory and secular terms^{7,10}.

We, and others¹²⁻¹⁴, attribute the seasonal variation in atmospheric CO_2 (about 6 p.p.m.) principally to seasonal changes in net photosynthesis and respiration of the biosphere, although purely physical and chemical criteria, such as annual variations in sea surface temperatures, may contribute slightly. The secular increase (about 0.8 p.p.m. yr⁻¹) is attributed to atmospheric retention of a fraction of the CO_2 produced by industrial processes (principally the combustion of fossil fuels, although the kilning of limestone and other anthropogenic activities contribute a small fraction). Nearly all industrial fuel burning occurs in the Northern Hemisphere.

Our procedure for the analysis of net hemispheric metabolism require the normalisation of the month-by-month CO_2 data by subtracting the industrially derived CO_2 . Since month-by-month global industrial fuel combustion numbers are not available, we estimate this by multiplying the global annual rates of anthropogenic CO_2 production by monthly proportions determined from patterns¹⁵⁻¹⁷ in the USA (Fig. 1b). These numbers are then corrected for the estimated rate of oceanic CO_2 uptake. The resulting curve, which would be the month-by-month pattern if there were no industrial additions during this time, is plotted in Fig. 1c.

We base our oceanic uptake corrections on the work of Broecker, who has estimated that as of 1970 the partitioning of industrial CO_2 among atmospheric and oceanic reservoirs is such that $58 \pm 7\%$ remains in the atmosphere¹⁸. His calculation is based on a model of the world's oceans which includes a thin, warm, mixed surface layer separated from the cold, deep ocean by a 1 km thick principal thermocline. His model also accounts for enhanced exchange of atmospheric carbon with the deep oceans in the cold, turbulent high latitudes.

Annual terrestrial net photosynthesis for each year is determined by subtracting the trough of each normalised annual curve from the preceding crest. Respiration is determined by subtracting each trough from the following crest. There is no direct precedent for determining net photosynthesis and respiration by this technique, although the method has conceptual forerunners in daily and seasonal net photosynthesis and respiration techniques well developed in the aquatic sciences and used occasionally in forest metabolism studies. The analysis would give us 'net ecosystem production' and 'net ecosystem respiration' as defined by Woodwell and Whittaker¹⁹, although they did not explicitly consider these definitions in terms of annual net growing and non-growing seasons, respectively, as we do.

Therefore we call our measurements 'net hemispheric metabolism', which includes 'semiannual net photosynthesis' and 'semiannual net respiration.'

A model of hemispheric metabolism that would give the observed curves (Fig. 1c) is shown in Fig. 2a. In a simulation of global metabolism, using twin sine waves as in Fig. 2a, we found our analysis to be sensitive to fairly small increases or decreases

in photosynthesis (defined as above) or similar changes in respiration (Fig. 2b), but simultaneous unidirectional changes in both photosynthesis and respiration were difficult to discern. But, as discussed below, we would not expect simultaneous unidirectional changes to occur. So we believe this analysis valid for discerning global metabolic trends.

We did not observe any significant trend in either 'semiannual net photosynthesis' or 'semiannual net respiration' for the period of this analysis (Fig. 1d). Linear regressions of both the photosynthesis (P) and the respiration (R) values were compared to a line of zero slope using Student's *t* tests. Neither regression line was different from the horizontal at the 50% level. Additionally, the slope of the linear regressions for the 15-yr trends in both photosynthesis and respiration, after correction for 51, 58 and 65% atmospheric retention of industrially derived CO_2 , never exceeded the standard error of the estimate of the slope for any of these lines.

We do, however, observe an increase in the PR ratio from 1962 through 1968, a change that would produce the dip seen in the calculated data of Fig. 2c. Since this change is not explicable by water temperature changes, we assume that this period was relatively favourable to photosynthesis and/or unfavourable to respiration. There was also some slackening in world food

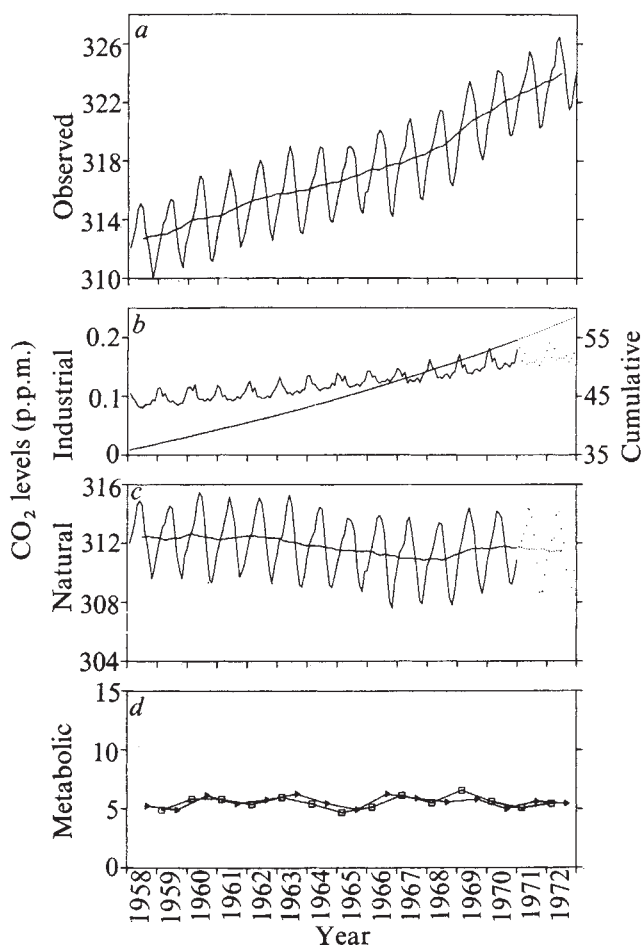


Fig. 1 a, Month-by-month variation in the CO_2 concentration of tradewind air at Mauna Loa, Hawaii. The upward sloping smooth curve is normalised for the semiannual fluctuations. b, The month-by-month addition of industrial CO_2 to the Northern Hemisphere, along with the cumulative production above the preindustrial level of 293 p.p.m. (ref. 25). The past two years are extrapolated values from the least square fit of data to a third-order polynomial. c, The observed CO_2 variation corrected for net industrial additions. d, Semiannual net ecosystem production and respiration, determined as the difference between the crests and troughs, in Fig. 2c. $\square$, Respiration by difference; $\triangle$, photosynthesis by difference. Oceanic uptake 42%.

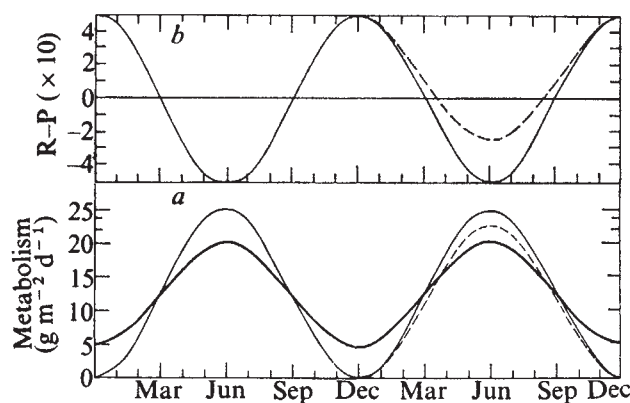


Fig. 2 *a*, A model of terrestrial biospheric metabolism extrapolated from the behaviour of the Brookhaven oak-pine forest^{12, 26, 27}. The difference between 24-h respiration (heavy line) and gross photosynthesis (lighter line) would produce the CO₂ fluctuations plotted as the solid line in Fig. 2*b* or Fig. 1*c*. A 10% reduction in gross photosynthesis would produce the patterns shown as dotted lines in Figs 2*a* and 2*b* superimposed on an upward secular trend as less CO₂ is removed from the air each year.

production during 1964 and 1965, although in general such annual correlations between our estimates of photosynthesis and world food production are not very strong.

There are several possible sources of error in our analysis. The most important include: an incorrect coefficient for the uptake of CO₂ by the sea; an underestimate of the quantity of CO₂ injected into the atmosphere by man's activities; and possible effects of annual or secular trends in oceanic surface temperature. We investigated each in turn by sensitivity analysis of our calculations and/or extensive calculations of work presented previously^{13, 20-22}. The results of these analyses (available from C.A.S.H.) strengthen our belief in the relative insensitivity of our principal conclusions to possible errors in the data base, and confirm our confidence in the numbers of Keeling, Rotty and Broecker.

Although there are several other factors that could interfere with our analysis that are not well understood at present or not measured, such as annual differences in Hadley cell or other atmospheric circulation patterns, we would expect any secular changes in metabolism to emerge above these, and show as a consistent increase or decrease in one of the lines in Fig. 1*d*, or in their relative positions. For example, a decrease in photosynthesis over a period of years probably would be accompanied by a decrease in the PR ratio, as decompositional processes continued to break down forest biomass. Similarly, if man were cutting forests significantly relative to net photosynthesis, the trees would be oxidised by way of decompositional pathways or burning, and the PR ratio would decrease. This is not consistently the case.

Therefore, we tentatively conclude that, although industrial processes have altered and often degraded many aspects of the biosphere, there is no evidence for changes in the basic pattern of Northern Hemisphere biotic metabolism from 1959 to 1972 within the limits of resolution by this method. Either the biosphere is too big to be affected yet or the potentially adverse effect of such things as acid rain, atmospheric dust loading, urbanisation or replacement of highly productive forests with less productive agriculture is approximately balanced by the stimulatory effects of atmospheric CO₂ enrichment and the increased use of fertilisers in agriculture³. In addition, the community compensatory mechanisms elucidated by Botkin and his colleagues²⁴ in computer simulations of forests would tend to damp potential changes. But if man's impact continues to grow at an exponential rate it will be interesting to follow the gross effects on the biosphere. We suggest our method and its

future refinements as one such global index, and recommend that other CO₂ monitoring stations be established at different latitudes and altitudes to facilitate this.

Part of this research was carried out at Brookhaven National Laboratory under the auspices of the US Atomic Energy Commission. The collection of the CO₂ data was performed under the auspices of the National Science Foundation. We thank D. Juers for statistical assistance, R. Houghton for ideas on forest metabolism, C. Cogbill for thoughts on tree-ring analysis, A. Hermann for help with the simulation and H. Lieth, R. H. Whittaker and G. M. Woodwell for critical comments.

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Volcanic influence on seawater at Heimaey

VOLCANISM is a contributing factor in marine geochemical processes. It has been shown to contribute to the dissolved silicon in seawater¹, it has also been suggested as the cause of some anomalous fluoride concentrations observed in the sea² and it has been seen as a possible source of manganese, iron and other elements found in unusual concentrations in manganese nodules and some deep sea sediments^{3,4}. Moreover, it has been suggested that in the geological past volcanism has affected the microfauna of large oceanic areas⁵. Here I describe the effect of the recent Heimaey eruption on the waters nearby.

The eruption on Heimaey, the largest of the Westman Islands, started on January 23, 1973, and lasted about five

Table 1 Chemical properties of surface seawater adjacent to the Heimaey lava

Sample no.	Date (1973)	T (°C)	S (‰)	SiO ₄ -Si (μg-atom l ⁻¹)	NO ₃ -N (μg-atom l ⁻¹)	F/Cl* (× 10 ³)	Mn (μg l ⁻¹)	Fe (μg l ⁻¹)	Zn (μg l ⁻¹)	Hg (ng l ⁻¹)	F (mg kg ⁻¹)
V 1	10.2		35.948	99.0	10.3	14.2	177		28.2		2.827
V 2	"		38.268	236	6.9	22.6	482		47.4		4.791
1	9.3	7.3	35.054	14.8	13.9	6.97	20.9	129	7.0	109	1.352
2	"	6.7	35.063	12.2	14.3	6.90	15.9	82.8	12.4	70	1.340
3	"	6.7	35.043	12.2	14.5	6.86	11.9	50.4	5.8		1.331
4	"	8.3	35.102	21.6	14.2	7.18	35.6	111	5.4	111	1.395
5	"	15	36.097	88.0	12.9	8.89	199	86.4	13.7	161	1.776
6	"	8.0	35.137	24.3	14.2	7.21	32.8	28.8	4.2		1.403
7	"	16	36.210	113	12.7	9.21	243	79.2	14.5		1.847
8	"	19	36.672	115	12.1	10.1	300	83.5	18.3	305	2.058
9	"	10.5	35.120	42.5	13.8	7.70	75.8	68.4	6.8		1.497
10	"	19	35.361	88.0	12.4	9.84	139	41.0	13.1	256	1.927
11	"	23	35.506	115	11.5	10.8	181	43.2	14.6	364	2.120
12	"	11	35.079	23.1	14.0	7.15	24.9	36.7	13.5	478	1.389
13	"	24	35.374	95.5	10.8	8.70	98.5	28.8	21.6	78	1.703
14	"	31	35.419	119	9.1	9.27	123	35.3	15.4	166	1.817
15	"	10.5	35.002	22.9	14.1	7.27	26.6	32.4	22.3	37	1.408
16	"	17	35.449	75.0	12.5	8.65	101	21.6	26.6	320	1.698
17	"	21	35.513	86.0	11.5	8.93	112	13.0	17.3	239	1.756
18	"	21	35.346	86.0	11.3	8.57	84.9	21.6	14.3	196	1.676
19	"	12.2	35.090	29.7	13.7	7.27	32.3	45.3	22.2	439	1.413
20	"	16	35.256	64.0	12.7	8.32	79.2	32.4	17.3		1.624
21	"	17	35.275	68.0	12.7	8.77	96.8	43.2	12.0	134	1.712
22	"	10.4	35.138	49.0	13.6	7.70	85.5	87.9	19.9		1.497
23	"	6.50	35.051	10.8	14.6	6.81	5.0				1.322
24	"	6.21	35.051	9.2	14.6	6.83	5.0				1.325
A 1	30.3	23	36.339	163		10.4	183	22.3	14.7	10.0	2.102
A 2	"	29	38.272	401		14.1	589	11.5	13.2	9.0	2.990
A 3	"	11.0	35.105	56.0		7.72	37.9	57.6	15.7	6.7	1.500
A 4	"	19	35.138	73.2		10.0	79.8	301	18.2	9.7	1.953
A 5	"	13.0	35.153	66.7		8.68	63.9	166	15.3	5.1	1.690
A 6	"	8.0	35.039	34.8		7.38	50.4	61.9	17.9	10.0	1.431

V1 and V2 samples, small volumes, collected from shore on the harbour side of the advancing lava. Samples 1–24 (Fig. 1) and A1 to A6 were collected from RV Bjarni Saemundsson and from a rubber dinghy by filling 4l polyethylene containers by immersion. The samples were subsequently split into subsamples. Trace metal samples, except mercury, were acidified with redistilled HCl. Mercury samples were collected from the rubber dinghy. Samples A1 to A6 came from the sheltered region off the northern lava front.

* Cl = S/1.80655.

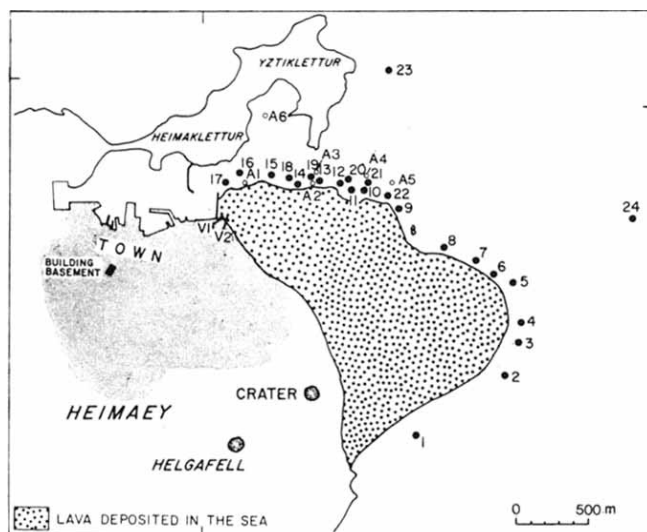
months, during which a substantial fraction of the volcanics was deposited in the sea⁶. Although the eruption did not occur under the sea it provided an opportunity to observe mobilisation of elements which resulted from the interaction between seawater and volcanics. The material for this study is listed in Table 1. In addition, the extent of the area affected and the normal regional conditions were assessed by examination of samples collected on February 10 during an oceanographic survey of RV Bjarni Saemundsson in the region of the Westman Islands. Temperature and salinity were determined as well as concentrations of fluoride⁷, silicate⁸, nitrate⁸, manganese⁹, iron¹⁰, mercury¹¹, zinc and copper¹².

At Heimaey, the influence of volcanism on the adjacent seawater arose mainly from the viscous lava that advanced spasmodically into the sea. This caused the temperature to rise and also, by evaporation, increased the salinity from the regional range of $S=35.0‰$ to $35.15‰$. On March 9 the warm surface layer that spread out from the lava front was generally thin with a thickness rarely exceeding 1 m. As might be expected this warm surface layer contained a concentration of dissolved silicon considerably in excess of the concentrations of $9\text{--}10\text{ }\mu\text{g-atom Si l}^{-1}$ found in the unaffected water.

The solubility and rate of dissolution of silicon in water increases with temperature^{13,14} and there is a significant positive correlation ($r=0.77$) between dissolved silicon and temperature (Table 1) and an even stronger correlation between dissolved silicon and salinity ($r=0.91$). Since the increased silicon concentration is the result of a dissolution process at the seawater–lava interface, it is of interest to relate the other constituents examined to the concentration

of dissolved silicon. All samples with significantly increased dissolved silicon concentrations also had enhanced fluoride/chlorinity ratio and there is a significant correlation ($r=0.75$) between the two parameters. This confirms that volcanism may considerably increase the fluoride/chlorinity ratio of seawater, although it is clear that such anomalies are unlikely near quiescent volcanoes^{14,15}. The fluoride added to seawater in the eruption may either be derived directly

Fig. 1 Seaward position of lava front and sampling locations on March 9, 1973. ▲, Samples collected February 10; ●, samples collected March 9; samples collected March 30.



from the gaseous emanations or be leached from the surface of the lava and tephra. A 100 mg l^{-1} fluoride solution has been obtained by leaching lava ejected on the first day of the Heimaey eruption¹⁶, and the close correlation between dissolved silicon and the fluoride/chlorinity ratio suggests that fluoride has been introduced chiefly by leaching.

The manganese concentration close to the lava front (Table 1) was much greater than the regional background concentration of $2\text{--}5 \text{ } \mu\text{g l}^{-1}$; it shows a strong correlation ($r=0.94$) with dissolved silicon (Fig. 2a). Comparison with rock analyses of the new Heimaey lava¹⁶, shows that whereas the elemental ratio by weight of manganese to silicon is 0.011 in the lava, the elements go into seawater solution in the ratio 0.058; this must leave the parent material impoverished in manganese. The distribution pattern of iron around the lava front is altogether different from that observed for manganese and there is no correlation with dissolved silicon or other enriched components. The greatest silicon and manganese concentrations and a very low iron concentration were found in a sample (A2) of seawater that had percolated through a lava front that had been stationary for some time but still remained warm. This suggests that manganese is more readily leached from the lava than iron. The highest iron concentrations on March 9 were associated with brownish patches observed in the sea; this was also the case on March 30. No attempt was made to separate colloidal size particles from samples and in the acidified conditions used for storage, colloidal ferric hydroxides will have redissolved. The distribution of iron suggests that the iron (II) leached from the volcanics was rapidly oxidised and that the colloidal hydroxides have partially separated from the enriched solution, probably by wave action, to create the patchy appearance. A differential precipitation mechanism of this type, that separates iron and manganese, has been anticipated¹⁷ and is thought to be the cause of the distribution of these metals in some deep sea sediments^{3,18}.

Thermal and redox conditions at the seawater-lava interface are important in the mobilisation of silicon, manganese and iron. For silicon the temperature is of greatest importance, but manganese and iron will only go into solution in reducing conditions. Iron in the fresh Heimaey lava has been shown to be rapidly oxidised in an atmospheric environment¹⁶. The greatest dissolution of iron and manganese would therefore be expected to occur when the lava front advances into the sea continually exposing new surfaces to attack by seawater. The importance of redox conditions was borne out in a simple experiment where tephra that fell on the RV Bjarni Saemundsson on March 9, 1973, was leached with seawater (1 g per 200 ml) for 15 min using a boiling

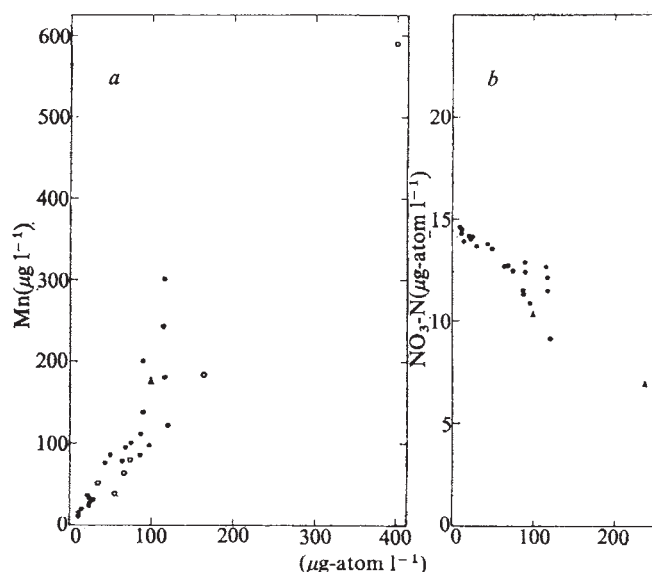


Fig. 2 Relationship between dissolved silicon and: a, manganese, b, nitrate nitrogen in the seawater near the lava front. ▲, Samples collected February 10; ●, samples collected March 9; ○, samples collected March 30.

water bath, the resultant solution gained $41 \text{ } \mu\text{g-atom Si l}^{-1}$, $6 \text{ } \mu\text{g Mn l}^{-1}$ and $4 \text{ } \mu\text{g Fe l}^{-1}$, but in a parallel experiment where the solution was 10^{-3} M with respect to hydroxylammonium chloride and slightly reducing, the gains were $41 \text{ } \mu\text{g-atom Si l}^{-1}$, $11 \text{ } \mu\text{g Mn l}^{-1}$ and $31 \text{ } \mu\text{g Fe l}^{-1}$.

The zinc concentration was relatively high in many of the samples enriched in silicon; however, there was no significant correlation between the two, which shows that zinc is not brought into solution in proportion to the silicon dissolved. Determinations of copper, cobalt and nickel were also carried out, but only in one instance was an increase above the normal background level observed; this was for sample A2 in which $16 \text{ } \mu\text{g Cu l}^{-1}$ was found—about three times the background.

The nitrate concentration decreases near the lava front with an inverse correlation to dissolved silicon, $r=-0.93$ (Fig. 2b). This somewhat unexpected finding might be explained as being the result of reduction of nitrate nitrogen to molecular nitrogen (or nitrous oxide) under the reducing conditions at the seawater-lava interface.

The extent of the volcanic influences on the chemistry of seawater was limited to the immediate neighbourhood of the eruption. The water temperature inside the harbour, which was initially 6.5°C did not rise above 25°C during the first four days of the eruption. During this time the northward extension of the eruptive fissure was active in the sea⁶. Samples from the oceanographic survey on February 10 revealed anomalies only at three stations that were at a distance between 2.2 and 4.6 km from the eruption. The anomalies were in temperature (up to 10.5°C), dissolved silicon ($26.6 \text{ } \mu\text{g-atom l}^{-1}$), manganese ($28 \text{ } \mu\text{g l}^{-1}$) and the fluoride/chlorinity ratio which attained a maximum 7.15×10^{-3} , compared with $(6.79 \pm 0.07) \times 10^{-3}$, for unaffected samples from this survey which is close to the normal oceanic ratio¹⁹ of 6.67×10^{-3} .

The mercury concentrations found near the lava front on March 9 (Table 1) were high for south Icelandic coastal water¹¹ and the highest concentrations do not correlate with either temperature or dissolved silicon. While collecting these samples it was twice observed that gas bubbles rose to the sea surface off the northern stretch of the lava front. Three weeks later, when the force of the eruption was

Table 2 Determinations of mercury in gas samples

Date	Sample	Volume sampled (l)	Mercury* ($\mu\text{g m}^{-3}$)
October 2, 1974	Reykjavik, air†	540	0.007
March 29, 1973	Heimaey town, street air	10	0.44
March 28, 1973	Heimaey town, building basement gas	1.2	3.34
		2.4	3.70
	Fumarole on south-west side of the crater, volcanic gas	1.2	15.0
		1.2	19.0
April 26, 1973	Chimney on lava lake, north east of the crater, volcanic gas	1.0	7.91
		1.0	10.8
		0.5	16.1

*Refers to volume of non-condensing gases.

†Collected by amalgamation on gold. Mercury in all other samples collected by drawing the gas sample with associated condensed water through silicon tube into a gas washing bottle containing 50 ml of 2% potassium permanganate in 25% sulphuric acid.

waning, no gas bubbles were noticed and the samples collected had low mercury concentrations (Table 1). Considering that the seawater mercury may be derived from the rising bubbles of volcanic gas, an effort was made to determine the mercury concentration in the latter. The results summarised in Table 2 show that in comparison with the normal air²⁰ from Reykjavík, the Heimaey values are high. On April 26 sampling conditions were particularly favourable near the crater and I believe that the gas then sampled represents the unmodified volcanic gas, but I am not certain whether this applies to the fumarole gas. The gas that issued into building basements and lower parts of the town was highly modified, usually containing more than 90% CO₂ (ref. 6). No comparable data are available for the concentrations of mercury in the gaseous emanations of erupting volcanoes, but geothermal mobilisation of the element is well known²¹. It is tempting to use the results in Table 2 to estimate the total amount of mercury ejected into the atmosphere in the eruption. For this purpose it is necessary to assume that the amount of both mercury and water vapour evolved during the eruption was proportional to the amount of solid ejecta, $250 \times 10^6 \text{ m}^3$, or about $600 \times 10^6 \text{ t}$ (ref. 6). Second, from solubility data²², an upper limit of 8% by weight is assumed for the water content of the magma that erupted from a depth of about 20 km (ref. 6). The most representative volcanic gas samples collected during the Heimaey eruption had an average water mole fraction of 63% (Bragi Arnason, personal communication). From the above and the average mercury concentration of $14 \mu\text{g m}^{-3}$ in the lava lake and fumarole gases, the upper limit for gaseous mercury emission in the eruption is $7 \times 10^9 \text{ g}$. This is a small quantity in comparison with the anthropogenic atmospheric input which has been estimated at $3 \times 10^9 \text{ g yr}^{-1}$ from coal combustion alone²³.

I thank all those who helped in the collection of water and gas samples.

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Received January 30; accepted March 26, 1975.

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Recognition of the oldest known fossil marsupials from Australia

A PRELIMINARY study has been carried out on fossil remains (unearthed in the British Museum) from a travertine deposit in Tasmania. Regardless of the precise taxonomic assignments ultimately given to this material, the evidence we present that a diverse fauna of diprotodont marsupials existed in Australia in

late Oligocene time is of considerable importance. This evidence gives tangible support to the hypothesis that marsupials have been residents of the Australian continent since the early Tertiary at least. The basic differentiation of herbivorous Diprotodonta from Marsupicarnivora (Ride, 1964) very likely took place before the separation of Australia from Antarctica. By late Oligocene time Tasmania was situated near 52°S latitude¹ bathed by warmer seas² and the travertine accumulated in an equable warm-temperate to subtropical environment supporting a rich forest vegetation^{3–5} the closest living equivalents of which occur in the uplands of New Guinea and New Caledonia. Present-day representatives of some of the fossil marsupials from the travertine still inhabit such tropical environments in northern Australia and New Guinea.

Allport⁶ has reported the discovery of fossil mammal remains in the travertine at Geilston Bay (42°50'S, 147°21'E) on the northern shore of the Derwent across the estuary from Hobart, Tasmania. At that time the travertine outcrops at the head of the Bay had been commercially quarried for over 20 years and were well known to naturalists for their rich content of fossil plant and animal remains. Allport noted that the bones we exhibited included forms similar to those of living potoroos and phalangers. Owen's report⁷ that "fragments of bones, some teeth, and ungual phalanges of a small kind of *Hypsiprymnus*, with probably also *Perameles* and *Phalangista*" had been identified, led Allport⁸ to state that the "bones all proved to belong to existing species", a conclusion that necessitated a considerable revision of the geological history postulated for the site. Allport was forced to explain how the bones of living species could be found many feet below the ground surface and beneath a basalt flow interbedded in travertine associated with a fossil flora having little in common with the present-day flora of Tasmania. Nothing more was written about the bones until Johnston⁹ intimated that "it is to be feared they have not been preserved". The Geilston Bay collection and its whereabouts thus passed into obscurity primarily because of the belief that it represented only a Recent fauna.

Mahoney, conducting a search for bibliographic materials relating to the Australian fossil mammals in the British Museum (Natural History), located the Allport collection in the museum's holdings. Preliminary examination of the fragmentary remains leaves no doubt that the fossils represent new taxa of marsupials. An assemblage of Tertiary age is strongly indicated, and this recognition has led us to re-examine the geology of the Geilston Travertine and associated basalts to determine the age of the fossiliferous deposit.

The bones were found⁶ in an "arenaceous clay, containing coarse grit, and a few slightly rounded pebbles" interbedded with the travertine. Matrix still adhering to some specimens confirms this description of the containing rocks. Allport also indicated that work west of the quarry towards the River Derwent had exposed the east dipping contact of the travertine with the overlying basalt. Several geologists have observed the quarry working face, for travertine was produced from the site at least until 1924. Johnston¹⁰ recorded (in descending order) the following composite section in the quarry and adjacent area: 1.5 m of weathered basalt overlying 1.2 m of silicified ostracod limestone, 1.8–2.4 m of yellow and brown mottled calcareous clay with marl lenses from which he alleged the marsupial remains were obtained, 3.1–3.6 m of travertine with plants and snails and 1.8 m or more of brown clay extending below the level of the estuary. A similar section, but without the basalt, was recorded by Krause¹¹ in his map of part of the Hobart area and Nye¹² reported "thin layers of limestone occur above the basalt" as well as beneath. Moore¹³ gives one of the last direct reports on the abandoned quarry and maps two outcrops of basalt along the south side of Geilston Bay, both of which are shown to extend to the edge of the estuary. Between these outcrops Moore's map shows "hornblende tuff", but this is a printing error, for the outcrops in that position are the Geilston Travertine (W. R. Moore, personal communication). Our observations show that the westernmost basalt

outcrop overlaps Permian (Malbina) siltstone at a higher elevation than shown on Moore's map, and that it extended beyond the head of the bay to become continuous with the eastern outcrop, and to thus overlap the Geilston Travertine. These relationships seem to indicate that the basalt partially filled a narrow canyon containing a spring which had built up a considerable travertine apron before being buried by basalt and which subsequently continued to deposit travertine above the basalt. The combined observations of many geologists extending over 100 yr clearly indicates that the Geilston Travertine is mostly sub-basaltic (that containing the marsupial bones certainly so) and that travertine deposition was halted for a time by the basalt incursion and it is thus reasonably contemporaneous with the basalt. These conclusions had been anticipated¹⁴.

It is now impossible (because of development) to secure a sample of the basalt at the travertine quarry. Therefore samples of the basalt from the western outcrop were collected for possible isotopic dating, and the least altered of these (Tasmanian Museum No. 22241; ANU No. 74-98) was measured by the K-Ar method. The sample was collected approximately 100 m from the head of Geilston Bay at sea level on the southern shore. This basalt contains about 5% mainly fresh olivine phenocrysts set in a well-crystallised fine-grained groundmass of plagioclase, clinopyroxene, olivine and iron oxide, together with 5% green mineraloid and minor calcite. The plagioclase shows evidence of some low temperature deuteric alteration. The basalt yielded an apparent age of 22.4 ± 0.5 Myr ($K = 0.765\%$; rad. $^{40}\text{Ar} = 3.07 \times 10^{-11}$ mol g^{-1} ; 100 rad. $^{40}\text{Ar}/\text{total } ^{40}\text{Ar} = 90.0$; $\lambda_e = 0.585 \times 10^{-10}$ yr^{-1} ; $\lambda_\beta = 4.72 \times 10^{-10}$ yr^{-1} ; $^{40}\text{K}/\text{K} = 1.19 \times 10^{-2}$ atom %). Because of the somewhat altered nature of the basalt the measured age must be regarded as a minimum estimate for the time of crystallisation because of the possibility of some diffusive loss of radiogenic argon. The measured age approximates closely the estimated age of 22.5 Myr for the Oligocene-Miocene boundary¹⁵, thus the Geilston Travertine underlying the basalt is considered to be late Oligocene or older.

The oldest previously known Australian marsupial *Wynyardia bassiana* was also from Tasmania at Fossil Bluff on the north-west coast where a skeleton was recovered from the Fossil Bluff Sandstone of early Longfordian age¹⁶. Recent radiometric calibration of the Longfordian Stage from the northern side of the Bass Basin in Victoria suggests that its base is approximately 21.4 Myr (ref. 17) and thus *Wynyardia* is of early Miocene age.

At least three mammalian taxa can be discerned among the very fragmentary remains from the Geilston Travertine. All identifiable remains seem referable to the Order Diprotodontia (Owen, 1866) and none can be relegated to the Peramelidae (Waterhouse, 1838) as Owen had stated. The largest form is represented by a fragment of a left maxillary with well worn dentition including part of M^1 and M^2 - M^4 complete (BMNH 40157, length M^2 - M^4 25.1 mm). This dentition resembles the Miocene palorchestine diprotodontid *Ngapakaldia* (Stirton, 1967) closely enough to encourage tentative assignment to that subfamily. It is about 60% of the size of *N. tedfordi* (Stirton, 1967) from the Ngapakaldi Fauna of the Lake Eyre Basin. More abundantly represented is a second and smaller form that is postulated on the basis of tentatively associated edentulous jaw fragments, an unworn left M^1 (BMNH 32000, length 4.3 mm) and left M^2 or M^3 (BMNH 32001, length 4.2 mm), and other tooth and skeletal fragments. In mandibular dental formula and morphology these remains resemble those of the smaller species of *Phalanger* (Storr, 1780) and although other features bar assignment to that genus, reference to the Family Phalangeridae (Thomas, 1885) seems likely. A third form is indicated by a lower incisor which agrees best with those of living *Burramyidae* (Broom, 1898).

We thank J. A. Mahoney (University of Sydney) and the trustees of the British Museum (Natural History) for permission to report on the Geilston fauna, and W. R. Moore for data on

the geology of the site. This research was supported in part by a NSF grant to R.H.T.

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Use of a self-made sound baffle by a tree cricket

FIELD observations and sound recordings of several South African species of *Oecanthidae* were carried out during the summer and autumn of 1973-74 to augment studies of the acoustic behaviour of the tree cricket performed abroad¹⁻⁶. We report that *Oecanthus burmeisteri* uses a leaf as a sound baffle to increase the intensity of its calling song by pressing its tegmina against the edges of a pear-shaped hole gnawed into the leaf. At least two more South African chirping *Oecanthus* species use a leaf baffle just as efficiently as *O. burmeisteri*.

The calling song of the male *Oecanthus burmeisteri* Sausure consists of regular chirps repeated for hours on end during the summer, from dusk to about 2 h past midnight. The chirping sound is actually produced by a frictional mechanism involving the elytra. The right front wing bears the stridulatory file, a row of regularly spaced 'teeth'. A sclerotised edge of the left wing serves as the scraper. When the file and scraper are rapidly rubbed together a sound is produced. Chirps recur at about 2 s intervals, each complete chirp lasting for 300 ms and consisting of ten bursts, each burst being made up of an almost sinusoidal oscillation at 2,000 Hz. This frequency is near the lower end of the scale for *Oecanthus* calls.

Interest in the sound production of the *Oecanthidae* was aroused by the first *O. burmeisteri* male found singing on a small sunflower leaf on December 21, 1973 in Pretoria. The insect producing the soft, insistent call was found on the underside of a leaf sitting in a small pear-shaped hole which it had gnawed and which differed from the irregularly shaped feeding holes. The foremost part of its body

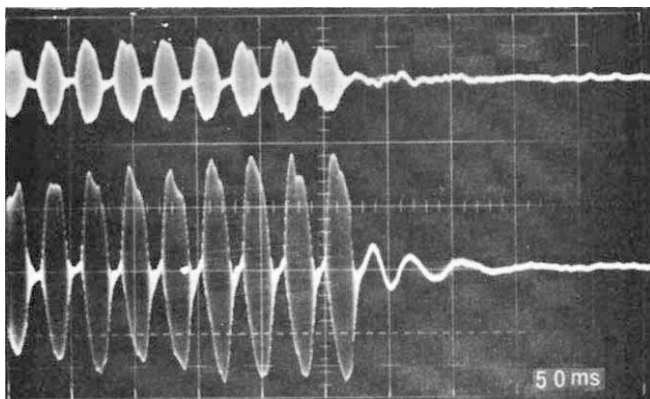


Fig. 1 Leaf used as a baffle. Wings pressed tightly against the edges of the hole gnawed by the insect.

and front legs protruded on to the upper leaf surface. During stridulation the elytra (or modified front wings) were raised at right angles and their lateral and apical margins were tightly pressed against the leaf surface surrounding the hole. Almost all male singers were sitting in similar positions.

Remembering that, according to Bennet-Clark⁷, the mole cricket forms its burrow into the shape of twin exponential horns to increase its sound intensity, it occurred to us that *O. burmeisteri* may be fashioning and using a leaf for the same purpose. A microphone was therefore placed at a fixed position 15 cm from a male and a recording made first of the insect's song when sitting in front of the hole and then immediately afterwards when this individual was sitting inside the hole with its wings pressed against the leaf (Fig. 1). The orientation of the insect with respect to the microphone was the same in both cases. Consequently there was no possibility that directional effects in the sound radiation pattern, as suggested by Bennet-Clark⁸, were present. Gain settings of the recording and reproducing systems were held constant.

Fig. 2 Oscillograms of two chirps of *Oecanthus burmeisteri*; upper trace, without baffle; lower trace, using baffle.



Oscillograms of the results obtained (Fig. 2) show that the sound amplitude is far greater in the second case, thus proving the effectiveness of the leaf baffle in amplifying the sound produced by stridulation. At the beginning of the chirp shown the amplitude ratio is 2.5 but rises to 3.5 for the final tone burst. This build-up is probably caused by the cricket steadily increasing the pressure of its elytra against the leaf, thus gradually increasing the effectiveness of the baffle.

A vibrating body, small compared with the wavelength of the sound it produces, is a poor sound radiator because a compression wave created on one side largely cancels the simultaneous rarefaction wave on the opposite side. For this reason Bennet-Clark⁸ as well as Michelsen and Nocke⁹ concluded that insects producing low frequency calls could increase their sound intensity by using a baffle. They show that as the size of a baffle placed around a sound source is gradually increased so the radiated sound intensity increases, reaching a maximum when the baffle diameter is approximately half the wavelength of the sound concerned. This is a well known acoustic phenomenon¹⁰.

The wavelength of the 2,000 Hz note of *O. burmeisteri* is 170 mm, the diameter of its sound-producing membrane approximately 3.2 mm—only one-fiftieth of a wavelength. Consequently the membrane is a poor sound radiator. The sizes of the leaves used ranged from 70×80 to 170×300 mm, so even the smallest formed an efficient baffle. The hole, usually near the centre of the leaf where it would be most effective, averaged 8×14 mm in size thus ensuring a good fit for the elytra.

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Measuring the relative importance of different pollinators to plants

THE development of a predictive theory of pollinator-plant coevolution requires a means of assessing and comparing quantitatively the costs and benefits of different pollinator foraging strategies and plant flowering strategies. Although there are quantitative data for pollinator strategies^{1–3}, the relative benefits to the plant of alternative pollinators, in terms of pollen transferred, ovules fertilised, and seeds set, have never been quantified for field conditions. Likewise, reference to the most effective pollinator⁴ of a plant species is often based on estimates of the abundance of alternative pollinators. Demonstrations that various pollinators can cause seed set in caged plants⁵ and that pollinators transfer pollen in different amounts^{6,7} are difficult to relate to natural conditions. We have developed a quantitative approach for comparing the relative importance of alternative pollinators in terms of the quantity, level of out-crossing and efficiency of pollen transferred. We have used this approach to investigate the pollination of

evening primroses, *Oenothera fruticosa* L. (Onagraceae), by two principal pollinators, European honey bees, *Apis mellifera* L., and soldier beetles, *Chauliognathus marginatus* Fab.

The quantity of pollen transferred per unit time can be calculated as the mean number of grains transferred to the stigma of a flower per pollinator visit multiplied by the mean number of flowers visited per unit time. Likewise, the quantity of pollen transferred per unit time by one species of pollinator can be calculated as the number of foraging individuals of that species multiplied by the quantity of pollen transferred by an individual per unit time.

Pollen transferred within a plant produces no seeds in self-incompatible species, and may result in lower quality seeds in self-compatible species. The proportion of pollen grains transferred that are out-cross (between plant) pollen (P) can be calculated considering the number of flowers visited per plant (N)^{8,9}, the number of pollen grains transferred per visit (G), and the number of grains on the body of the pollinator (B):

$$P = (1/N) \sum_{i=1}^N (1 - G/B)^{N-1} \quad (1)$$

We assume that the pollen load on the pollinator is in dynamic equilibrium and that an equal number of pollen grains is exchanged on each visit.

The relative importance of soldier beetles and honey bees in the pollination of *O. fruticosa*, a self-incompatible plant, was investigated in a moist field north of Durham during May and June 1973 and 1974. The yellow flowers with eight stamens and a prominent, four-lobed stigma, remain open for 1 or 2 d (between approximately 0900 and 1700). Bees visited the flowers primarily for nectar, while beetles foraged for both nectar and pollen. Bees averaged 4.0 s per flower and beetles averaged 27.6 s per flower, during 341 and 54 visits, respectively. Over this time, the bees visited 4.9 flowers per plant whereas the beetles visited 2.6 flowers per plant. Bees and beetles were caught as they left the flowers and pollen was removed from them in agitated alcohol rinses. The bees averaged 1,100 pollen grains on their bodies ($n = 12$; s.d. = 494; range 436–1730) while the beetles averaged 217 ($n = 8$; s.d. = 190; range 19–570).

To estimate the pollen grains transferred per visit, virgin flowers were presented to the foraging bees and beetles and the grains on the stigma were counted after one visit. Bees transferred a mean of 193 pollen grains per visit ($n = 24$; s.d. = 135; range 9–548), while beetles transferred 114 grains per visit. The latter figure is based on only two observations, but for illustrative purposes the data for beetle pollen transfer will be used in further calculations. Individual bees and beetles transferred 174,000 and 14,900 pollen grains per hour, respectively. If the number of foraging bees and beetles is equal, then the former account for 92.1% of all pollen transferred. This estimate is biased in favour of beetles, as there seemed to be many more bees than beetles in any given area of the *Oenothera* population.

The level of out-crossing effected by the pollinators can be calculated from equation (1). For bees carrying 1,100 pollen grains, transferring 193 grains per visit, and visiting 4.9 flowers per plant, 71.2% of the pollen transferred would be out-cross pollen. For beetles carrying 217 grains, transferring 114 grains per visit, and visiting 2.6 flowers per plant, 63.5% of the pollen transferred would be out-cross pollen. Thus, bees would account for 92.9% of the out-cross pollen being transferred.

The energetic efficiency of pollen transfer was contrasted for the two pollinators on the basis of quantity of pollen transferred per calorie of nectar available. *O. fruticosa* flowers produced 0.3 µl of nectar at 45.3% sucrose equivalence or 0.52 calories per flower averaged across the population. Assuming that the pollinator removed all nectar from the flower during a visit, bees transferred 373 pollen grains per calorie of nectar pro-

duced, and the beetles 220 pollen grains per calorie. With a mean of 143 ovules produced per plant and a seed set of 54% averaged across the population, nectar production can be expressed as 0.036 calories of nectar per ovule produced or 0.067 calories per seed set. Individual bees transferred more pollen grains per unit time, more efficiently, than the beetles and a greater percentage of those pollen grains was out-cross pollen resulting in higher seed set. On the other hand, beetles may be able to forage profitably on smaller plants or poorer quality flowers because of their low level of activity and presumed lower caloric requirements. In sparse populations of *O. fruticosa* or on days when only a few flowers are open, beetles may increase in their relative importance as pollinators.

Differences in the pollination service provided by various pollinators may be an important selective force operating on plant populations. If pollinator service limits seed set, genotypes that possess flowers more attractive to a certain pollinator or a more efficient pollen attaching or removing mechanism will produce more seeds¹⁰. Alternatively, if pollinators are abundant, genotypes which receive the same pollinator service per flower while producing less pollinator attractants per flower, may be able to produce more flowers and set more seeds. Evolutionary shifts in floral morphology, colour and timing of flower production¹¹ are almost certainly associated with a new pollinator providing a higher quantity of pollen transfer or a higher level of out-crossing than a previous pollinator. The new pollinator may also provide the same level of pollen transfer but require less food during foraging. The honey bee as an introduced pollinator may impose a high selection pressure on *O. fruticosa* in populations where it is the primary and most efficient pollinator. Although insufficient time may have elapsed from introduction for the honey bee to have had an impact as a selective force on the particular set of characters that *O. fruticosa* now possesses, a responsive shift in floral characters may be predicted in the future. Only by measuring both the importance of pollinators to plants and the importance of plants to the pollinators, can the reciprocal selective forces which operate at the animal-plant interface be determined.

We thank F. Almeda, J. Antonovics, M. Berenbaum, B. Heinrich and D. E. Stone for suggestions. The study site was made available with the permission of John Blue. The authors were supported as NIH predoctoral trainees by a US Public Health Service training grant.

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Received February 10; revised March 28, 1975.

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¹⁴C-ethylene incorporation and metabolism in pea seedlings

ETHYLENE is now generally accepted as an important, integral part of the natural hormonal complement of many plants¹. Although the morphological, physiological and biochemical effects of the gas are well known, surprisingly little is known about its metabolism. There is no agreement as to whether or not ethylene is even incorporated into plant tissues. Several reports^{2–4} of work using ¹⁴C or ³H-labelled ethylene indicate that it is not incorporated whereas others^{5–10} suggest that a small, yet significant amount is fixed and metabolised. The

criticism^{1,11,12} of these papers, on the basis that adequate precautions were not taken to insure that the label fixed and metabolised was derived from ethylene, has led Abeles¹ to conclude that ethylene incorporation is probably not a prerequisite for ethylene action. A second major criticism of these studies is that no precautions were taken to exclude microbial contamination which could have accounted for the observed metabolism. Here, I report the results of experiments in which ^{14}C -labelled ethylene of very high purity was applied to etiolated pea seedlings under aseptic conditions to study its possible incorporation and metabolism. They reveal a previously unrecognised active metabolic system in pea which converts $^{14}\text{C}_2\text{H}_4$ to $^{14}\text{CO}_2$, as well as to unidentified tissue components.

and 0.8 ml was counted in a similar manner. All data were corrected for counting efficiency and sample quenching by the internal standard method using ^{14}C -hexadecane.

A highly significant and readily detectable amount of $^{14}\text{C}_2\text{H}_4$ incorporation and conversion to $^{14}\text{CO}_2$ was observed in pea seedlings when treated with $15\ \mu\text{l l}^{-1}$ of $^{14}\text{C}_2\text{H}_4$ (Fig. 2a). During the first 20 h after imbibition, the amount of $^{14}\text{C}_2\text{H}_4$ incorporated and converted to $^{14}\text{CO}_2$ by the pea seedlings was relatively small, amounting to 1.1 and 0.6 d.p.m. per mg dry weight per 20 h, respectively. During day 2, however, tissue incorporation and $^{14}\text{CO}_2$ release rapidly increased reaching a peak on day 3 of 12 and 33 d.p.m. per mg dry weight per 24 h, respectively, and then gradually declining during days

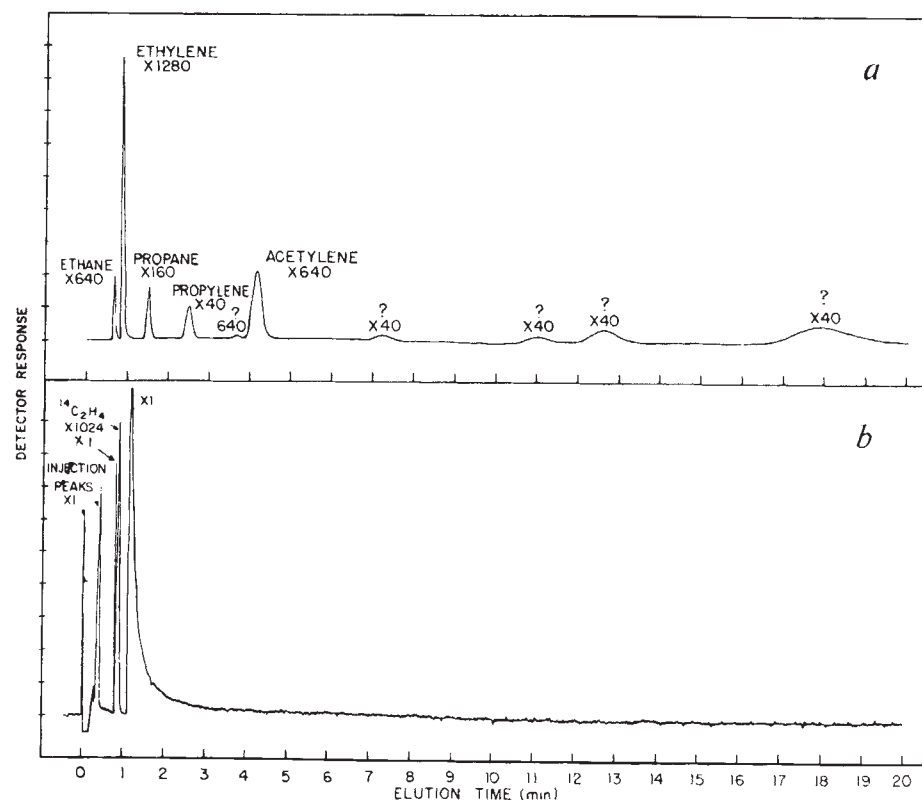


Fig. 1 Gas chromatograms of $^{14}\text{C}_2\text{H}_4$ (specific activity $53.0\ \text{mCi mmol}^{-1}$) before (a) and after (b) purification. Samples analysed on an activated alumina column using standard flame ionisation gas chromatography. This system could detect 6 parts per $10^9\ \text{C}_2\text{H}_4$ in a $1\ \text{cm}^3$ sample. At least nine major impurities were detected in the purchased $^{14}\text{C}_2\text{H}_4$ before purification whereas none was present in the twice purified material. The two $^{14}\text{C}_2\text{H}_4$ peaks at retention times of 0.80 and 0.85 min are the result of attenuating the $^{14}\text{C}_2\text{H}_4$ peak at 0.80 min from maximum sensitivity ($\times 1$) to $\times 1,024$ to prevent it from going off scale. The apparent peak at 1.1 min is the result of increasing the sensitivity back to $\times 1$ and represents the tailing of the $^{14}\text{C}_2\text{H}_4$ peak at this higher sensitivity.

Uniformly labelled $^{14}\text{C}_2\text{H}_4$ (reported radiochemical purity 98%, 22 or 53 mCi mmol^{-1}) was purified twice by gas chromatography (GC) and first separated on a silver nitrate ethylene glycol-coated Gas Chrom R column¹¹ at 0°C . The ethylene fraction was collected and further purified using the same technique on a Poropak T column. Figure 1 illustrates the effectiveness of this purification scheme. No impurities were detected in the purified $^{14}\text{C}_2\text{H}_4$ even at maximum GC sensitivity.

With a gas-tight syringe fitted with a Millipore filter ($0.22\ \mu\text{m}$), the desired amount of purified $^{14}\text{C}_2\text{H}_4$ was immediately injected into flasks containing three aseptically grown etiolated pea seedlings (0–5 d old). Each flask contained 3 ml sterile deionised water and 1 ml 1.5 N NaOH (electrolytic pellets) in the centre well. The flasks were placed on a shaker for 24 h in the dark at 27°C . All operations were conducted using a 'safe' green light. Following incubation the ethylene concentration in each flask was determined and the seedlings plus the 3 ml of water were transferred aseptically to a mortar and homogenised. An aliquot (0.1 ml), diluted 20-fold, was plated on each of four different culture media to check for microbial contamination. Petri dishes were incubated at 30°C for 1 week and the data from any sample showing contamination were not used. The remaining homogenised tissue was lyophilised, weighed, resuspended in water and counted by liquid scintillation in Aquasol (New England Nuclear). The NaOH was removed

4 and 5. A peak of natural ethylene production preceded the peak in ethylene metabolism by approximately 24 h (Fig. 2b) and like ethylene metabolism gradually declined thereafter. In contrast to ethylene production and metabolism, respiratory CO_2 production remained relatively constant after reaching a peak of $17\ \mu\text{l}$ per mg dry weight per 24 h on day 4 (Fig. 2c).

Considering the rigorous purification procedures used, it was highly unlikely that an impurity in the labelled ethylene may have still accounted for these results. Nevertheless, additional evidence was sought to further eliminate this possibility. The purified material was first pretreated with NaOH to remove possible acidic impurities or with $\text{Hg}(\text{ClO}_4)_2$, KMnO_4 or $\text{Br}_2\text{-H}_2\text{O}$ to remove ethylene before exposure to pea seedlings. The results are presented in Fig. 3 and indicate that the incorporated radioactivity is derived from $^{14}\text{C}_2\text{H}_4$. Treatment with base had no effect whereas treatment with all three ethylene trapping agents eliminated the observed effect. Furthermore, regeneration of the $^{14}\text{C}_2\text{H}_4$ from the specific ethylene complexing reagent, $\text{Hg}(\text{ClO}_4)_2$ (ref. 13) completely restored activity.

Ethylene metabolism in these seedlings is almost certainly biological since essentially no radioactivity was recovered in the tissue or as $^{14}\text{CO}_2$ when the seedlings were heated to 80°C for 1 min before $^{14}\text{C}_2\text{H}_4$ exposure or when the $^{14}\text{C}_2\text{H}_4$ was applied in anaerobic conditions. Additional evidence supporting this view, as well as indicating the complexity of the ethylene

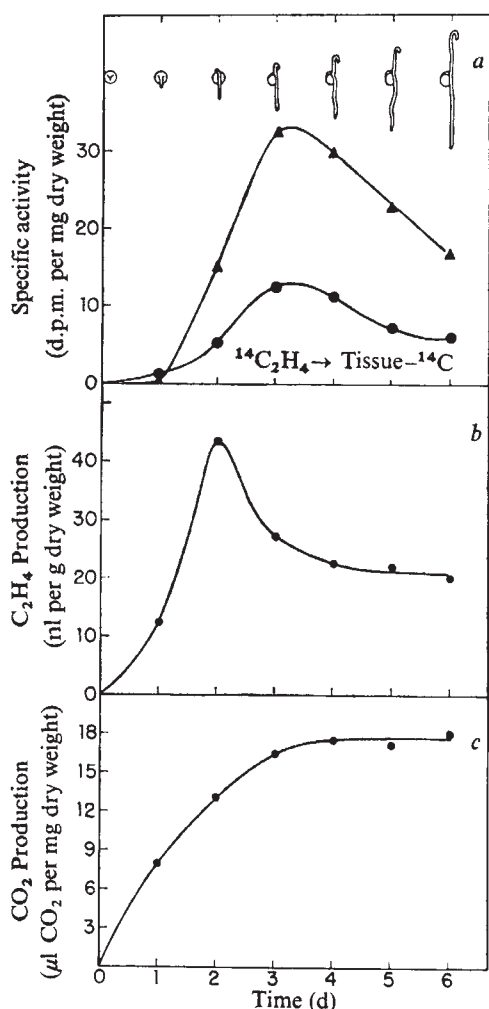


Fig. 2 *a*, Time course of $^{14}\text{C}_2\text{H}_4$ conversion to $^{14}\text{CO}_2$ (▲) and $^{14}\text{C}_2\text{H}_4$ incorporation into tissue components (●) of aseptically grown pea seedlings; *b*, natural ethylene production; *c*, natural CO_2 production. $^{14}\text{C}_2\text{H}_4$ specific activity was 22 mCi mmol $^{-1}$. Each datum point is the average of nine seedlings. Three such experiments were conducted with similar results. Pea seeds (*Pisum sativum* L. cv. Alaska) were rinsed with water and ethanol and surface-sterilised for 15 min in 1% sodium hypochlorite. The seeds were rinsed with 4 l of sterile water, treated for 10 min with 0.01 N HCl (ref. 14), rinsed again and placed in the dark in an equal volume of sterile water for 4 h at 27 °C. Following imbibition the seed coats were removed aseptically and the seeds rinsed four times with 4 l of sterile water. Sterilised seeds were germinated at 27 °C in the dark in Pyrex dishes between moist sheets of filter paper. Seedlings were transferred aseptically into 62 cm 3 flasks with glass centre wells and ground-glass stoppers. The freshly purified $^{14}\text{C}_2\text{H}_4$ was injected through a side-arm fitted with a 5 mm rubber serum stopper and the arm was immediately sealed off from the rest of the flask by rotating the stopper, as volatile substances from the serum stoppers often caused erratic results. Natural C_2H_4 and CO_2 production were determined in the same conditions as in (*a*) except that no $^{14}\text{C}_2\text{H}_4$ was added. CO_2 production rates were measured by liberating the CO_2 from the NaOH with acid followed by gas chromatography to determine the amount released. CO_2 production rates in conditions in which 10 $\mu\text{l l}^{-1}$ ethylene was added were only slightly higher than in air. The radioactivity, recovered in the NaOH was identified as $^{14}\text{CO}_2$ by liberating the CO_2 from the NaOH with acid and then isolating the CO_2 fraction by GC and comparing the radioactivity present in this fraction with the amount originally present in the NaOH. s.d. for $^{14}\text{C}_2\text{H}_4$ to $^{14}\text{CO}_2$ conversion (▲) in (*a*) for days 1 to 6 were $\pm 0.7, 2.6, 5.8, 5.2, 1.4$ and 0.4 , respectively. The s.d. for $^{14}\text{C}_2\text{H}_4$ incorporation into the tissue (●) in (*a*) were $\pm 0.2, 1.1, 2.4, 0.8, 0.3$ and 0.2 , respectively.

metabolism system, is the fact that ethylene metabolism was reduced over 80% if the cotyledons were detached from the seedlings during incubation of the whole seedling.

These experiments demonstrate that ethylene is incorporated and metabolised by intact pea seedlings. As yet, the possible physiological significance of this observation in terms of ethylene action is not clear. It is of considerable interest, however, that a peak of natural ethylene production should precede the peak in ethylene metabolism. This sequence does not seem to be the result of a delay in the peak of ethylene metabolism caused by a reduction in growth since the same sequence was observed in other experiments (E.B., unpublished) involving much shorter exposure periods in which the added ethylene had no detectable effect on growth. It is possible that ethylene action depends on its permanent incorporation into cellular components. Perhaps the peak in natural ethylene production and the peak in ethylene incorporation and metabolism reflects the progress of senescence in the cotyledons. The level of incorporation observed in these experiments could have been observed only through the use of $^{14}\text{C}_2\text{H}_4$ of high specific activity. I have found that CO_2 , which often acts antagonistically to ethylene (ref. 1), will severely inhibit conversion of C_2H_4 to CO_2 . This finding further suggests that ethylene metabolism and action may be closely linked.

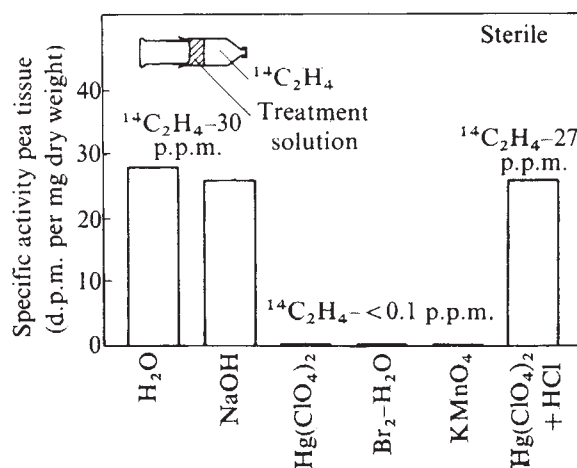


Fig. 3 The effect of pretreating purified $^{14}\text{C}_2\text{H}_4$ (specific activity 22 mCi mmol $^{-1}$) with water, NaOH, $\text{Hg}(\text{ClO}_4)_2$, $\text{Br}_2\text{-H}_2\text{O}$ or KMnO_4 on its subsequent incorporation into aseptically cultured 2 d old pea seedlings. $^{14}\text{C}_2\text{H}_4$ (3 ml, 900 $\mu\text{l l}^{-1}$) was pretreated for 30 min over 1 ml of each treatment solution in a 5 ml glass syringe. The gas phase (2 ml) was then injected into a flask containing three pea seedlings and the seedlings were incubated for 24 h at 27 °C. Each datum point is the average incorporation of nine seedlings. The $\text{Hg}(\text{ClO}_4)_2$ solution was one-fifth the concentration recommended in ref. 13, thus eliminating the formation of impurities 8 .

In view of the relatively large amounts of impurities found in all of the $^{14}\text{C}_2\text{H}_4$ samples purchased, it is difficult to assess the significance of previous studies. The instability of labelled ethylene is illustrated by the fact that within one week of purification, labelled radiolysis products could be detected. Previous studies 5,9,10 are further complicated by the fact that $^{14}\text{C}_2\text{H}_4$ was routinely regenerated from $\text{Hg}(\text{ClO}_4)_2$ before exposure to plant tissues. As reported by Jansen 8 and confirmed in similar experiments, this procedure leads to the formation of impurities which are readily incorporated into plant tissues. These criticisms raise considerable doubt as to the validity of previous studies and their significance must be evaluated on the basis of more critical experiments.

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Received December 9, 1974; accepted March 27, 1975.

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Table 2 Mean body weight and mean fluid intake ($\pm$ s.e.)

Strain	Group*	N	Body weight (g)	Water intake (ml g ⁻¹ d ⁻¹)	Alcohol intake (ml g ⁻¹ d ⁻¹)†	(g kg ⁻¹ d ⁻¹)‡
C57BL	1	7	12.7 $\pm$ 0.7	0.036 $\pm$ 0.013	0.303 $\pm$ 0.032	22.7 $\pm$ 2.4
C57BL	2,3	18	12.0 $\pm$ 0.3	0.090 $\pm$ 0.015	0.174 $\pm$ 0.013	13.1 $\pm$ 1.0
DBA	4	10	11.6 $\pm$ 0.4	0.189 $\pm$ 0.040	0.113 $\pm$ 0.029	8.5 $\pm$ 2.2
DBA	5,6	19	10.9 $\pm$ 0.4	0.267 $\pm$ 0.021	0.056 $\pm$ 0.009	4.2 $\pm$ 0.7

*Refers to groups of Table 1.

†ml of 7.5% (w/v) ethanol per g per d

‡g of ethanol per kg per d.

Alcohol selection by DBA and C57BL mice arising from ova transfers

THE notion of the heritability of alcoholism has alternated between acceptance and rejection¹⁻⁴. Convincing evidence for a genetic involvement in man has been reported^{5,6}, but the issue remains controversial⁷. Because nature and nurture are so intertwined, and the methodology of their separation in humans is difficult, and even often suspect, inbred mouse strains have been used to examine the genetic basis of the disparate voluntary alcohol intakes seen in these strains^{8,9}.

The transfer of fertilised ova between strains of mice exhibiting extremes of alcohol choice separates the contributions of heredity and environment. Measurement of the retention, or the loss, of strain-typical preference or aversion for alcohol in the offspring arising from fertilised ova transferred to a mother of a different strain then enables an estimate to be made of the relative contributions of heredity and environment to alcohol selection. Our findings uphold the hereditary basis of alcohol selection in C57BL mice, but show the avoidance of alcohol drinking by DBA mice to be amenable to environmental alteration.

Ova donors and recipients were primiparous adult female C57BL/6 (alcohol preferers) and DBA/2 (alcohol avoiders) mice born in our colony from breeder stock purchased from Jackson Laboratories. The mice were housed in plastic cages (19 $\times$ 29 $\times$ 14 cm) covered with a wire-mesh top indented to hold Purina lab chow and cylinders of fluid graduated to 0.2 ml. A cycle of 12-h light and 12-h dark was used. Fifty-four offspring were used as subjects; their grouping and sexual composition are shown in Table 1.

Ova were transferred by a modification of the method of McLaren and Michie¹⁰. Donor females were used 3.5 d and recipient females 2.5 d after identification of a vaginal plug.

At birth, the litters were culled to a maximum of four pups, since no more than three transfers were born to one female; the remaining pups, ranging from one to three, were her own. Differences in eye pigment identified experimental pups at birth. In the case of control transfers (C57BL to C57BL and DBA to DBA), litters were culled to a maximum of four without respect to sex. The untreated controls were mated and housed in the same manner as the experimental and control transfer animals, but subjected neither to anaesthesia nor to surgery.

Pups were weaned at 25 d and housed individually for 7 d; during this time they were offered a choice between tap water

and 7.5% ethanol (w/v, prepared from 95% ethanol). The fluids were presented in two 25-ml cylinders graduated to 0.2 ml and fitted with stoppers and ball-bearing spouts. Daily fluid consumption was measured and the tubes were alternated; the subjects were weighed every other day. A mean preference ratio (ml 7.5% w/v ethanol per ml of total fluid per d) and mean fluid intake (alcohol, water and total fluid per g body weight per d) were calculated for each subject based on the 7-d test period.

Since no sex differences were noted in either strain, the sexes were pooled and treated as one; the two control groups for each strain (untreated mice and transfers to the same strain) were not statistically different from each other and these data were also combined for analysis. The group means ($\pm$ s.e.) for the various measures are shown in Table 2.

An analysis of variance for a complete orthogonal set of contrasts for the transfer and strain variables was carried out. Strain differences were highly significant for body weight, water and alcohol intake, preference ratio, and g ethanol per kg per day ($F = 5.73-65.2$, d.f. = 1/50, $P = < 0.000001-0.021$). C57BL mice weighed more, drank more alcohol and less water than the DBA mice. Collapsing across strains, treatment condition (transfer compared with non-transfer) was significant for water and alcohol consumption, preference ratio and g ethanol per kg per day ($F = 7.04-22.59$, d.f. = 1/50, $P = 0.000017-0.011$). Control mice of both strains drank less alcohol and more water than transferred mice. The strain $\times$ treatment interaction was significant only for the water, alcohol and g of ethanol per kg per day measures ($F = 4.29-10.26$, d.f. = 1/50, $P = 0.0024-0.044$). Mice arising from transferred ova of both strains thus drank less water and more alcohol than their respective control groups. No differences in total fluid intake were noted.

Our results support previous findings^{8,9} that alcohol preference in the C57BL strain has a genetic basis, but provide even more conclusive evidence. Offspring arising from drinker ova transferred to the uterus of a non-drinker mother and raised by her not only retained, but increased their strain-typical high alcohol preference. We cannot offer a convincing (factually based) rationale for this increase. The offspring arising from fertilised non-drinker DBA ova transferred to drinker C57BL mothers also increased their alcohol intake and thus failed to retain their strain-typical aversion to alcohol, suggesting that either the prenatal or postnatal environment, or a combination of the two, is sufficient to increase alcohol consumption in DBA mice. Support for a positive postnatal rearing effect has been reported by Komura *et al.*¹¹ in normal (non-transferred) mice and we have confirmed¹² this; DBA offspring fostered by C57BL mothers from birth to weaning increased their voluntary alcohol consumption, but in C57BL weanlings (pups fostered by DBA mothers) alcohol intake was unaffected. These results do not, of course, preclude the possibility that the prenatal environment is of equal importance: a cross-fostering study of offspring from transferred ova would be required to partition accurately the maternal effects.

Our results support the genetic basis of alcohol selection in the C57BL mouse, the nature of which is still unknown, while demonstrating that alcohol avoidance in DBA mice is subject

Table 1 Distribution of subjects among experimental groups

Group	Male	Female	Donor	Recipient	Condition
1	5	2	C57BL	DBA	Experimental
2	3	5	C57BL	C57BL	Control
3	6	4	—	—	C57BL untreated control
4	4	6	DBA	C57BL	Experimental
5	4	6	DBA	DBA	Control
6	7	2	—	—	DBA untreated control

to environmental manipulation. The identification of those physiological and/or biochemical factors under genetic control and determining alcohol preference should appear high on any list for investigation.

Although research on the genetic aspects of alcohol preference in mice may serve to further the understanding of the predisposing role of inherited factors, any results garnered from such research cannot easily be generalised for their applicability to the problems of alcoholism in man.

We thank Dr Benson Ginsburg and Ms Alice Trottnier (University of Connecticut) for help with the surgical procedure. This research was supported by grants from the Busch Memorial Fund (predoctoral fellowship to C.L.R.) and a biomedical sciences support grant from Rutgers University, the US National Institute on Alcohol Abuse and Alcoholism and the Distilled Spirits Council of the United States. C.L.R. is an NIAAA Postdoctoral fellow.

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Dominance at *Adh* locus in response of adult *Drosophila melanogaster* to environmental alcohol

It has been argued¹ that natural selection acting on enzyme polymorphisms may be most clearly demonstrated by challenging polymorphic populations with an environmental additive acting as a specific substrate for the enzyme under study. Wills and Nichols², for example, showed that, in their experimental conditions, heterozygote advantage at the octanol dehydrogenase locus in *D. pseudoobscura* depended on the presence of octanol in the food medium. Similarly, de Jong *et al.*³ found that the amylase^{4,5} variant increased strikingly in frequency in populations of *D. melanogaster* moved from a sucrose to a starch-rich food medium. Amylase^{4,5} is known to possess the highest *in vitro* activity on starch substrates of all common amylase variants⁴. We have examined the relationship between mortality and genotype at the alcohol dehydrogenase

(*Adh*) locus when adult *Drosophila* are exposed to environmental ethanol.

Wine cellars in Spain support extremely large populations of *D. melanogaster* that feed on, and breed in, the fermenting and maturing liquor (12-15% ethanol) impregnating floating mats at the surface of wine jars. Neighbouring rubbish tips and grape-skin composts also sustain populations. It seems probable that all the *D. melanogaster* within a town, and perhaps within a much larger region, form a single breeding unit, as we have observed considerable migration between bodegas (wine cellars). Recent studies of lethal allelism have indicated that samples of *D. melanogaster* collected at sites as much as 1.4 miles apart may belong to the same population⁶. We would therefore attribute any local differentiation of allele frequencies to selective values differing between sites, rather than to the sites representing independent discrete populations.

As might be expected, the *Adh*^F allele, whose enzyme product has a higher ethanol catalytic activity per individual than that of the *Adh*^S alternative, is at a high frequency (0.94-1.00) in cellar populations throughout Spain (D.A.B., J-M.M., and A.R., unpublished). The *Adh*^S allele is more favoured in non-cellar environments, being at a significantly higher frequency in a rubbish-tip sample than in our standard (Mancha) wine cellar, although the two sites are less than 1 km apart (Table 1). Similarly, the frequency of *Adh*^S increases dramatically in laboratory populations bred from large (~10⁴ adults) samples removed from wine cellars (D.A.B., J-M.M., and A.R., unpublished).

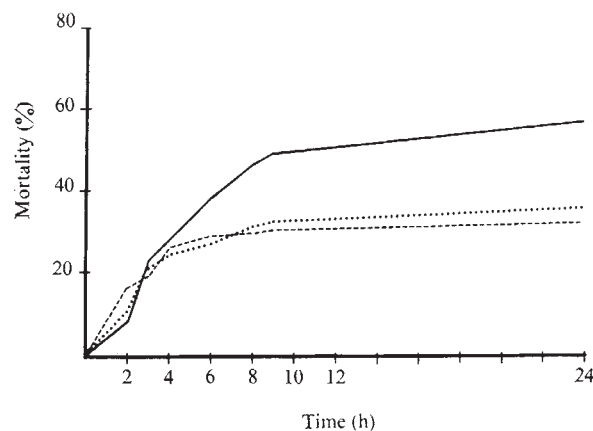


Fig. 1 Mortality/time plots for the three *Adh* genotypes when adult *D. melanogaster* are allowed to feed on medium containing ethanol (12.5%). —, *Adh*^S/*Adh*^S; ····, *Adh*^F/*Adh*^S; ---, *Adh*^F/*Adh*^F.

We have tested the proposition that alcohol is the major environmental factor maintaining a high frequency of *Adh*^F in wine cellars by exposing adult flies to ethanol in a situation which mimics that confronting *Drosophila* entering a cellar and feeding on wine. Flies from the Mancha and Mandila population cages (which had been initiated 18 and 6 months previously with samples from two cellars 200 km apart), and from the standard Kaduna laboratory population, were placed in beakers. A Petri dish containing food (Edinburgh medium⁶ supplemented with ethanol to a concentration of 12.5%) was fixed to the mouth of each beaker. The food was replaced every 2-3 h to maintain the concentration of ethanol. The *Drosophila* readily fed on this medium. Flies which died were removed and immediately electrophoresed to determine their *Adh* genotype. All individuals which survived were removed at 24 h and electrophoresed. No death occurred in controls in which the medium was supplemented with distilled water.

A typical mortality-time profile is shown in Fig. 1. Mortality is expressed as a percentage [(number of individuals of a given genotype dead after a given time/total number of that

Table 1 *Adh* genotypes among wild-caught *D. melanogaster* from a wine cellar and neighbouring rubbish tip

	<i>Adh</i> genotype			Frequency of <i>Adh</i> ^F ± s.e.
	<i>Adh</i> ^F / <i>Adh</i> ^F	<i>Adh</i> ^F / <i>Adh</i> ^S	<i>Adh</i> ^S / <i>Adh</i> ^S	
Wine cellar	177	15	0	0.961 ± 0.010
Rubbish tip	152	37	3	0.888 ± 0.016*

*The two samples differ significantly $P < 0.001$.

Table 2 Mortality (%) of the three *Adh* genotypes after 24 h exposure to ethanol food

Population	<i>Adh^F/Adh^F</i>	<i>Adh</i> genotype <i>Adh^F/Adh^S</i>	<i>Adh^S/Adh^S</i>	Increase in frequency of <i>Adh^F</i> allele
Mandila (starved)	33.2 (211)	34.5 (258)	54.8 (62)	+0.0269
Mancha (starved)	43.2 (229)	45.6 (237)	74.5 (51)	+0.0430
Mancha (prefed)	12.4 (145)	16.5 (133)	36.4 (22)	+0.0192
Kaduna (starved)	68.2 (110)	69.9 (103)	91.7 (24)	+0.0612
Kaduna (prefed)	25.3 (99)	32.1 (109)	45.8 (24)	+0.0278

Total numbers of each genotype in the sample are given in parentheses.

genotype in the sample) $\times 100$]. It is clear that mortality occurred predominantly during the first few hours of exposure. Accordingly the results for all samples have been expressed as total mortality (%) for each genotype after 24 h on ethanol food (Table 2). These data demonstrate a clear differential mortality between the genotypes, the *Adh^S/Adh^S* homozygote being significantly less resistant to alcohol poisoning than the heterozygote or *Adh^F/Adh^F* homozygote. The resulting increases in the frequency of *Adh^F* (frequency in survivors minus frequency in the sample before treatment) are given in Table 2. Increasing the rate of feeding, and hence of alcohol uptake, by starving the flies for 24 h in a humid chamber before treatment, significantly elevated the overall mortality.

In view of the strong selection against *Adh^S* in the presence of ethanol it is surprising that this allele is present at all in wine cellars. Presumably some form of balancing selection acting at other stages of the life cycle, or gene exchange with non-cellar populations, maintains the low frequency. Note that *Adh* alleles are not in linkage equilibrium with respect to other loci in Spanish populations, *Adh^S* being held in an inversion together with α -*Gpdh^F* (D.A.B., J-M.M., and A.R., unpublished). Selection for or against *Adh^S* therefore acts on the entire contents of the inversion, and conversely, selection at other loci within the inversion will influence the frequency of *Adh^S*. Nonetheless, we consider that the observed mortality differences between genotypes are due primarily to the *Adh* locus, and not to selection acting on a linked locus, as the results from the Spanish populations are strictly comparable with those from the Kaduna population, which lacks inversions and detectable linkage disequilibrium.

McKenzie and Parsons⁷ report that Chew failed to detect significant gene frequency differences at the *Adh* locus between Australian wine cellars and neighbouring non-cellar sites, and discount the alcohol dehydrogenase (ADH) system as a component of alcohol tolerance in *D. melanogaster*. Our results lead us to an opposite conclusion. If the treatment described above is a reasonable reflection of a natural situation we must conclude that the *Adh* phenotype expressed in an individual fly is an important component of its ability to tolerate environmental alcohol and, likewise, that adult mortality in the presence of ethanol-rich food plays a major role in maintaining a predominance of the high-activity *Adh^F* allele in wine-cellar populations.

Table 2 also shows that, in terms of survival on ethanol, the *Adh^F* allele is effectively dominant over the *Adh^S* alternative. This is at first sight surprising as all published data, and our own unpublished work, are in agreement that the *in vitro* ADH activity of *Adh* heterozygotes does not deviate consistently from the mid-point between the two homozygotes⁸⁻¹¹. For example, the ratios of the mean specific activities of the *Adh^F* homozygote, heterozygote and *Adh^S* homozygote in the Mancha and Kaduna populations are 3.25:2.24:1.00 and 1.91:1.41:1.00, respectively (D.A.B., J-M.M., and A.R., unpublished). Additivity at the primary gene-product level but dominance at the physiological level are, however, the general observations, where data on inborn errors of metabolism are available^{12,13}. Further, this relationship has been shown to be a general expectation derived from theoretical analyses of enzyme systems (ref. 14 and J. A. Burns, and H. Kacser, unpublished) and the data presented here support that prediction.

Dominance may influence the maintenance of a polymorphism in two ways. First, directional selection in a single environment becomes increasingly ineffective as the recessive allele becomes rare, thus dominance protects the recessive allele from extinction when a population encounters an environment in which the recessive is at a disadvantage. Secondly, should the dominance relationships be reversible under alternative conditions within a temporally or spatially varying environment, a marginal heterozygote advantage may result which could actively maintain the polymorphism.

We thank Norma Alexander for assistance. This work was supported by the ARC and the Juan March Foundation.

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Received February 4, 1975.

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Maintenance of allozyme polymorphisms in experimental populations of *Drosophila*

A CONTROVERSIAL point in modern population genetics is whether allozyme variation is adaptive or neutral. The interpretation of gene frequency distribution in terms of components of the environment¹⁻⁴ for a given species, or in terms of the comparison of patterns of allelic variation among different species^{5,6}, are cited as evidence of selection. Support for the alternative hypothesis derives from the apparent agreement between models based on neutrality and empirical findings either for the distribution of amino acid substitutions in proteins^{7,8} or for the distribution of heterozygosity⁹. These arguments have been challenged on theoretical grounds^{10,11}, as well as on the basis of the distributions of allelic frequencies found in natural populations^{12,13}.

The selective value of allozyme polymorphisms may be assessed by testing allozyme systems under experimental conditions. We have used this method to study several experimental populations and look for changes in allozyme

frequencies through the generations. Results reported here indicate that selection is probably responsible for the changes in gene frequencies observed in our experimental populations. These changes are, however, also influenced by the adaptive value of the genetic background that interacts with the allozyme genotypes under study.

The loci under study were *Est-5* (X chromosome), *Mdh-2* (IV) and *Odh* (II) in *Drosophila pseudoobscura*. For the *Est-5* locus, 19 independent homozygous strains were obtained by brother×sister matings from the progenies of wild females collected recently at McDonald Ranch (Coast Ranges of Northern California). For the *Mdh-2* and *Odh* loci we similarly obtained 31 and 19 independent strains, respectively. This represents a large number of independent chromosomes sufficient to avoid any kind of large artificial linkage disequilibrium at the beginning of the experiment.

For each of these three enzyme loci two population cages (A and B) were started each with about 3,000 individuals. The founder individuals were obtained from the strains mentioned above, combined in appropriate numbers to start one cage (A) with a high initial frequency (0.80) of the rare naturally occurring allele (respectively, 104, 112 and 104 for *Est-5*, *Mdh-2*, and *Odh*), and another cage (B) with a low initial frequency (0.20) of that allele. The experimental populations were maintained and sampled at regular intervals for more than 600 d. Figure 1a–c shows the results. Gene frequencies have changed significantly in every one of the A cages, but the B cages do not show evidence of significant changes. The two *Est-5* cages may have attained a common equilibrium level, but this result must await future generations of sampling for confirmation. Most significant is that all the changes in the A cages are in the same direction, namely towards the natural gene frequencies.

In every one of the A cages there was a large initial drop in the frequency of the rare allele. As a result of the rarity of this allele in nature, the original homozygous strains for this rare allele were more inbred than the homozygous strains for the commonest allele, and this may be the cause of the low initial fitness values of the genotypes with the rare allele. To test this possibility we crossed the original homozygous strains for the *Est-5*¹⁰⁴ in pairs, using each strain in two crosses, as the male parental in one cross and as the female parental in a different cross. The F₁ progenies of these crosses were again crossed in pairs and this procedure was continued for five generations, to obtain a unique strain still homozygous for *Est-5*¹⁰⁴ but with a largely heterozygous genetic background. This same procedure of matings was used with the strains homozygous for *Est-5*¹⁰⁰. With these two outcrossed strains we started two new population cages with high (cage 80) and low (cage 20) allele frequencies, similar to the A and B cages described above. Figure 1d shows the changes in gene frequencies observed during the first 500 d of sampling. The initial drop for allele *Est-5*¹⁰⁴ of cage 80 is similar to that of cage A (Fig 1a); this rules out the effect of inbreeding as a major factor responsible for the initial drop of the rare allele in the original cages.

An interesting feature of the results (Fig. 1d) is that the *Est-5*¹⁰⁴ allele now seem to be levelling off at a higher frequency than it did previously in cage A. *Est-5* strains had been kept in the laboratory for one year (about 12 generations) before the serial crosses were started to obtain the outcrossed lines. Under the prolonged overcrowded environment of the cultures one might expect selection to favour genes which increase genotype efficiency to compete and to exploit this kind of environment (K-selection). Adaptive changes caused by modifier genes have been reported not only in allozymes^{15,16} but also in morphological mutants^{17–20} caused by directed selection or in response to laboratory conditions. We propose that selection for these modifier genes associated with the *Est-5* locus may have occurred during this time, and that this may be a major reason for

the increase in fitness shown by the genotypes with *Est-5*¹⁰⁴ allele.

Yamazaki²² did not find significant selective differences between two alleles of *Est-5* locus in *D. pseudoobscura*. The flies used in his experiment, however, were extracted from an experimental population cage which had been previously kept in laboratory conditions for many years. These

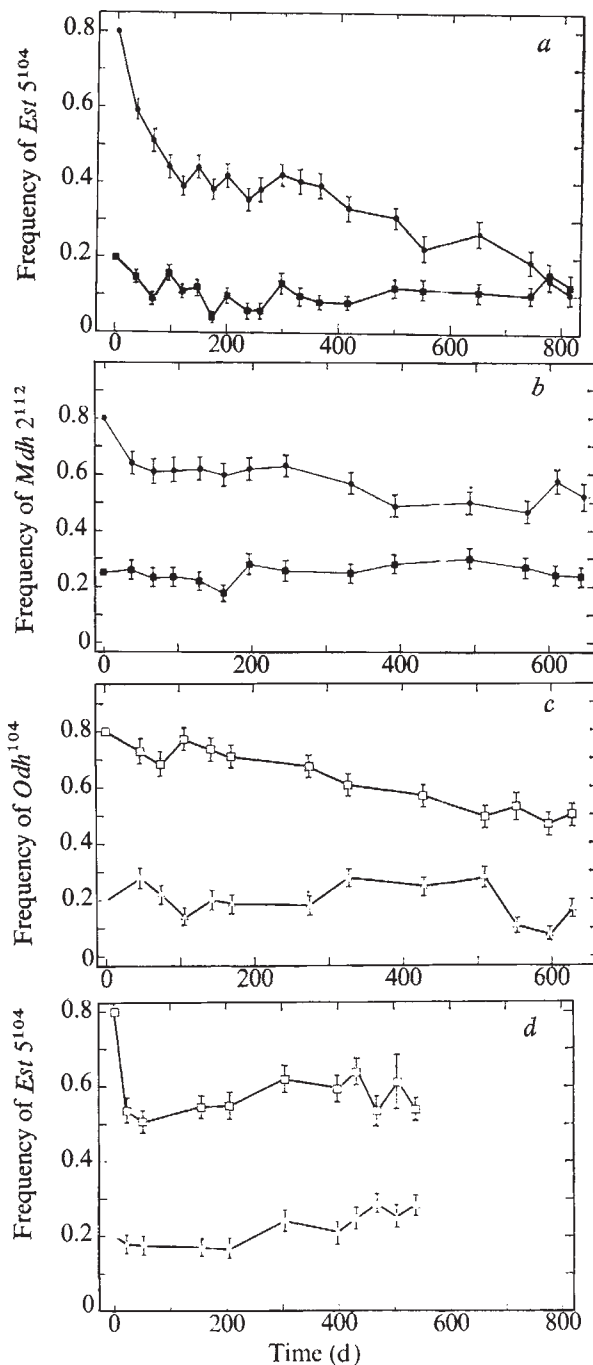


Fig. 1 a, Mean frequencies (95% confidence intervals) of allele *Est-5*¹⁰⁴ of *D. pseudoobscura* in two experimental population cages, A (●) and B (■). The other allele present in the cages is *Est-5*¹⁰⁰. Each point is based on two samples of about 150 eggs taken from each cage during 3 consecutive days. Each individual sample of eggs was placed in a separate culture from which we sampled at random 24 emerged females. The genotypes of these females (a total of 144 per cage per sample) were analysed for the *Est-5* locus using starch gel electrophoresis²⁴. b, Allele *Mdh-2*¹¹² in cage A (●) and B (▲). The other allele present in the cages is *Mdh-2*¹⁰⁰. Sampling as in (a) except that the total number of flies subjected to electrophoresis was about 96 per sample. c, Allele *Odh*¹⁰⁴ in cage A (□) and B (△). The other allele present in the cages is *Odh*¹⁰⁰. Sampling as in (b). d, Allele *Est-5*¹⁰⁴ in two experimental cages, 80 (□) and 20 (△). Sampling as in (a).

populations may well have built up a high adaptation to the experimental conditions through the selection of highly adapted favourable gene modifiers. The presence of these modifiers may obscure the detection of small differences in fitness between *Est-5* alleles.

The fact that selection is not operating exclusively and directly on allozymes but also simultaneously on other interacting genes does not rule out selection as responsible for the maintenance of allozyme polymorphisms. The uniformity of response in all the initial cages (A, B) in the present experiment towards frequencies close to the naturally occurring ones, is not what would be expected if the allozymes were neutral and associated randomly with other genes subjected to selection. If this were the case, one should find a variety of responses²¹.

Marinkovic and Ayala²³ have tested several fitness components for the same genotypes we used and their results are consistent with the view that selection is operating on the three loci we studied. Our results also indicate that selection must be acting on these loci, if not directly, at least through a series of coadapted gene complexes in which the allozymes genes are integrated.

We thank Miss Lorraine Barr for assistance. This work was supported by a US Public Health Service international fellowship, by a contract from the US Atomic Energy Commission and by a fellowship from the Ministry of Education, Spain.

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Received February 5; accepted March 27, 1975.

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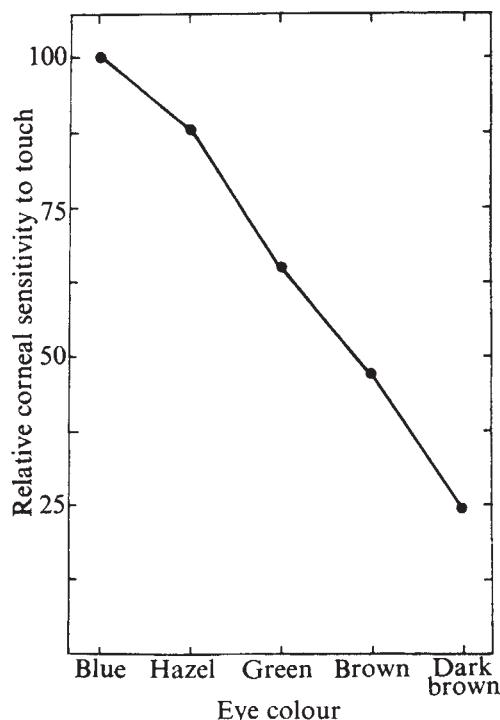
menon, I carried out an experiment in which corneal sensitivity to touch could be compared among people with different eye colour.

The Cochet-Bonnet aesthesiometer¹ was used to stimulate the cornea. Two models of the instrument, with nylon monofilament of 0.12 and 0.08 mm diameter, were used to produce pressures from 11-200 mg/0.0113 mm² and from 2-90 mg/0.005 mm², respectively. Both aesthesiometers are interchangeable and had previously been calibrated. The aesthesiometer was mounted in a holder such that it could be moved in x, y and z axes by means of three knobs. Thus, it was possible to achieve reliability in stimulation of a corneal point, a steady speed of application and a perpendicular corneal contact. A corneal point near the limbus in the six o'clock position was stimulated and the slightest bend of the nylon wire visible through a magnifier was defined as corneal contact. The inferior corneal point was chosen because the touch threshold measured there is not affected by psychological factors².

Measurements of corneal touch thresholds were made subjectively³. The experiment began with stimulation of the cornea with the lowest pressure and continued in an ascending fashion. At each predetermined length of the nylon monofilament (with increments equal to 0.5 cm with each instrument), four to six contacts were made with at least one blank to test the subject's reliability. The subject indicated when he felt the probe by pressing a bell. From these measurements the corneal touch threshold was defined as the length of the monofilament at which the subject responded for 50% of the number of stimulations. This length was converted into pressure using a previously calibrated curve for the instrument. The tolerances for this test are within $\pm 5\%$ as evinced by repeated measurements on the same subjects.

Caucasian subjects (112) 21-37 yr old (mean 23.5) participated in this experiment. Another group of 44 non-white subjects 22-33 yr old (mean 24.2) comprising 12 Negroes, 15 Indians and 17 Chinese, also served as subjects. As all these people (except four) were university students, they were about the same age in each group. All subjects were free of ocular pathology. The

Fig. 1 Relative corneal sensitivity to touch as a function of the colour of the eye. The dark brown group consists of the non-white subjects. The sensitivity is the reciprocal of the touch threshold measured in mg mm⁻² and the group with the greatest sensitivity has been assigned 100% in this diagram.



Do blue-eyed people have more sensitive corneas than brown-eyed people?

It has sometimes been noted by opticians involved in the fitting of contact lenses that the sensitivity of the cornea seems to vary, depending on whether the patient has blue or brown eyes. Since it is likely that the sensitivity of the cornea reflects general tactile sensitivity, this knowledge could be valuable. Because there does not seem to be any quantitative report of this pheno-

Table 1 Corneal touch threshold (mg mm⁻²) in people with different iris colour

	Blue	Hazel	Green	Brown	Dark brown Indians	Negroes	Chinese	Total dark brown
No. of subjects	39	12	12	49	15	12	17	44
Mean	14.3	16.2	22	30.4	47	61.6	64.5	57.8
S.d.	4.7	7.4	13.3	19.0	14.8	22.5	21.2	20.8

subjects were separated into groups according to the colour of the iris: blue, hazel, green, brown and the darker brown eyes of the group of non-whites.

The corneal touch thresholds obtained in variable order with these people are given in Table 1. These values were converted into sensitivity (threshold⁻¹) and the greatest sensitivity value was assigned 100%. The relative corneal sensitivity to touch for the various groups is illustrated in Fig. 1, in which there is only one group of people with dark brown eyes representing the non-white subjects.

The results of this study provide strong evidence that people with blue irises have the most sensitive corneas and people with brown eyes least sensitive; the sensitivity further diminishes in non-whites with darker pigmented eyes. It can also be seen (Fig. 1) that blue-eyed people have, on average, twice as sensitive corneas as white people with brown eyes (difference statistically significant, $P < 0.001$). The mean corneal touch threshold (and standard deviation) of all Caucasian females ($n = 55$) was 23.91 mg mm⁻² (s.d. 16.01) and of all Caucasian males ($n = 57$) 21.06 mg mm⁻² (s.d. 14.9). The difference between sex is not significant ($P > 0.5$), although it is worth noting that females are slightly less sensitive than males by about 10%. This factor may be accounted for by the fact that women have much less sensitive corneas for about 3 days at about the time of menstruation compared with the rest of the month⁴.

It also seems from the present study that non-white people who have dark brown irises have much less sensitive corneas than white people. The difference between the group of white people with brown eyes and the non-white is significant ($P < 0.001$). The present results show that non-white people have, on average, four times less sensitive corneas than blue-eyed people and half as sensitive corneas as whites with brown eyes.

It could be argued that the variations displayed here were caused by the fact that corneal sensitivity varies diurnally⁵ but this is unlikely as the diurnal variation is considerably smaller than the differences between eye colour.

This phenomenon is obviously relevant to the wearing of contact lenses. It may also have some bearing on problems of anaesthesia as it is possible that different quantities of anaesthetics may be needed according to eye colour. It may be inferred that corneal sensitivity reflects the general sensitivity of the body; and there is some evidence supporting this view as it was found that at about the time of menstruation corneal sensitivity is reduced⁴, and the touch sensitivity of the middle finger is diminished⁶.

This hitherto unreported phenomenon may help to explain large variations in corneal sensitivity in normal subjects. This variation can be puzzling when comparing the effect of some treatment or disease on the eye against a group of control subjects; this has already been noted in a study⁷ of corneal sensitivity in diabetics.

It is already a known clinical fact that, in the eye, more drugs are needed on people with dark rather than with light-pigmented irises to obtain the same effect (for example cycloplegia). The reason for this is not known although it may be related to the amount of melanin pigment present in the iris, where ocular drugs have an effect. Since the cornea does not contain any such pigment, however, it is not easy to explain the diminution of corneal sensitivity with darker pigmentation of the iris. It is not known whether the thickness of the cornea varies with eye colour. It has been shown⁸ that thicker corneas, as a result of oedema, are less sensitive than normal corneas. It is also unknown whether nerve density varies widely among these

different people. An alternative possibility is that the difference in sensitivity may arise in the central nervous system and not at the periphery. The difference in tactile sensitivity may also be relevant to understanding that the practice of acupuncture may be more acceptable in China than it is in countries inhabited by blue-eyed people.

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Iris colour and relationship of tyrosinase activity to adrenergic innervation

IRIS colour to some degree depends on the sympathetic innervation of the eye. Chance clinical observations of humans and experimental studies in animals have demonstrated consistently a gradual lessening of colour intensity in the iris after interruption of the sympathetic pathways to the eye^{1,2}. Similarly, full iris pigmentation fails to develop when the sympathetic innervation to the eye is absent from birth³. Interest in the relationship of sympathetic innervation to iris colour has increased with reports of direct adrenergic innervation to iris stromal melanocytes⁴⁻⁶. For mammals this is extraordinary in that most melanocytes, for example those of the skin, have no innervation. We have investigated this relationship using tyrosinase activity as an index of melanin synthesis since melanin is the chief pigment of the iris and tyrosinase is the rate-limiting enzyme in the production of melanin⁷. In turn, iris tyrosinase activity was measured in normal rabbits and in rabbits in which the sympathetic pathways to the eye have been interrupted.

Twenty-eight brown-eyed, Dutch belted rabbits of either sex with an average weight of 2.3 kg were used. In 10 animals the superior cervical ganglion on one side was resected under direct visualisation whereas in 18 animals the preganglionic nerve trunk was interrupted. To distinguish between the two types of operation the terms denervation and decentralisation are commonly used⁸. The occurrence of ptosis and miosis postoperatively was accepted as evidence that surgery had been successful. Survival times varied from 1 to 370 d. In all animals that survived longer than 2 months, the iris on the operated side became noticeably lighter in colour than that in the other eye.

Tyrosinase assays were performed in duplicate according to a modification of the method of Pomerantz⁹. Iris tissue, either homogenised or intact, was placed in a solution containing sodium phosphate buffer, 0.01 M, pH 6.8, and L-tyrosine-3,5-³H, 10⁻³ M, specific activity 50 Ci mol⁻¹. The mixture was incubated at 37 °C for 2-5 h. At the end of the reaction period, the material was treated with Norit SG to remove all tritiated substrate as well as tritiated reaction

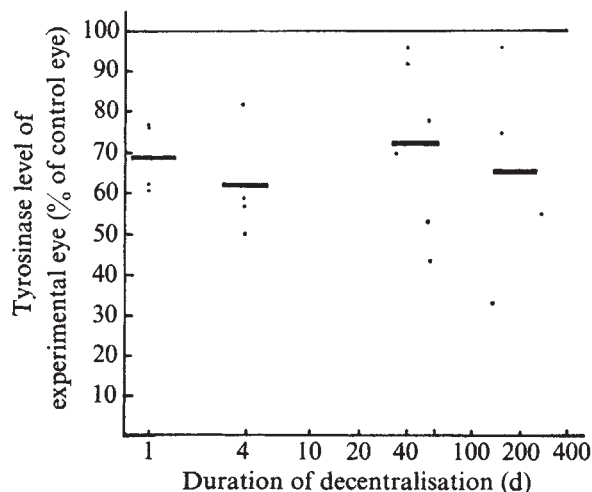


Fig. 1 After decentralisation of the superior cervical ganglion, iris tyrosinase activity declines rapidly. Once diminished, tyrosinase activity continues at the new, lower level for many months.

products except for tritiated water. An aliquot of the tritiated water was counted in a liquid scintillation counter. The activity of samples from iris tissue was calculated on the basis of c.p.m. per mg wet weight of tissue. Blanks for the assays consisted of either the reaction mixture without iris tissue or the reaction mixture plus iris tissue from an albino eye.

After either decentralisation or denervation, enzyme activity decreased in the ipsilateral eye. The results for both sets of animals are summarised in Table 1. The magnitude of the decline in iris tyrosinase was the same irrespective of surgical procedure. It should be remembered that the iris not only contains innervated iris stromal melanocytes but also has a posterior double lining of densely pigmented epithelial cells. Thus the proportion of the decline of iris tyrosinase activity that is attributable only to the iris stromal melanocytes cannot be told from these studies.

When the assay results were plotted to show tyrosinase activity as a function of duration of decentralisation (denervation is similar but not illustrated here) (Fig. 1) we found that enzyme activity in the iris decreased within 1 d and remained depressed for at least 200 d. Further, the assays of tyrosinase activity in the choroid (the posterior part of the uvea) in the same eyes yielded a similar rapid and long lasting decrease in enzyme activity.

In the face of such a clear-cut result, it seems reasonable to suggest a failure of maintenance, that is, to ascribe the lightening of iris colour that follows interruption of the sympathetic pathways to the eye to the inadequate replenishment of melanin within iris stromal melanocytes. Thus as the melanin of the iris is slowly depleted by normal metabolic processes, the iris gradually loses colour. Although there is no electron microscopic evidence at present for metabolism of iris melanin¹⁰, there has been a gradual build-up of information concerning degradation of melanin-

containing bodies (melanosomes) in epidermis¹¹ and in certain preparations *in vitro*¹².

In its dependency on adrenergic innervation, ocular tyrosinase is not unique; it has become clear that an intact innervational network is a prerequisite for the regulation of enzyme levels in several organs innervated by the sympathetic nervous system. Perhaps the most interesting example of this type of regulation occurs in the pineal gland where under the influence of noradrenaline, levels of *n*-acetyltransferase can increase thirtyfold in a few hours¹³⁻¹⁴.

We thank John Hendee, Maria Smith and Barbara Briggs for technical assistance. This work was supported by grants from the US Public Health Service, Research to Prevent Blindness, Inc. (to A.M.L.) and the American Cancer Society (to A.B.L.).

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Received February 3; revised March 24, 1975.

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Adult thymectomy results in loss of T-dependent mitogen response in mouse spleen cells

THERE is overwhelming evidence that murine thymocytes represent a heterogeneous population of cells that can be divided into discrete subpopulations on the basis of surface antigen markers^{1,2}, drug sensitivity³, physical characteristics^{4,5} and responsiveness to mitogens⁶. There is some indication that a similar heterogeneity with regard to biological function exists within the peripheral thymus-derived (T) lymphocyte population (reviewed in refs 7-9). Several investigators have shown that thymectomy of young adult mice reveals a differential sensitivity of several T-cell activities to this surgical procedure and suggested the existence of T-cell subsets with different life spans⁷⁻⁹. Therefore we investigated the response to PHA and concanavalin A in adult thymectomised (ATX) mice to determine if responsiveness to these mitogens could be attributed similarly to subpopulations of T cells.

We report here that spleen cells from BDF₁ mice thymectomised when 4 weeks old exhibit an initial increase in T-dependent mitogen responsiveness over age and sex matched littermate sham-operated controls (STX) followed by a rapid and sustained decay of responsiveness to these same mitogens, and that the response of identical cell preparations to a thymus-independent B-cell mitogen, lipopolysaccharide (LPS), does not show this divergence of responsiveness when thymectomised animals are compared with controls.

Of five experiments carried out, all gave similar results, and Fig. 1 shows the results of a representative experiment.

Table 1 Effects of operation on iris tyrosinase activity

		Operated side as % of control side
Denervation*	Mean	70.0
	s.e.	5.4
Decentralisation†	Mean	67.4
	s.e.	4.2

* *n* = 10.

† *n* = 18.

Adult thymectomy initially caused an increase in the response of spleen cells to phytohaemagglutinin (PHA) and con A. This was soon followed by a precipitous decrease in the level of con A and PHA responsiveness of cells from ATX animals while those from STX mice continue to increase to their mature adult level of responsiveness.

The response to pokeweed mitogen (PWM) (not shown here) was also lower in ATX animals although the

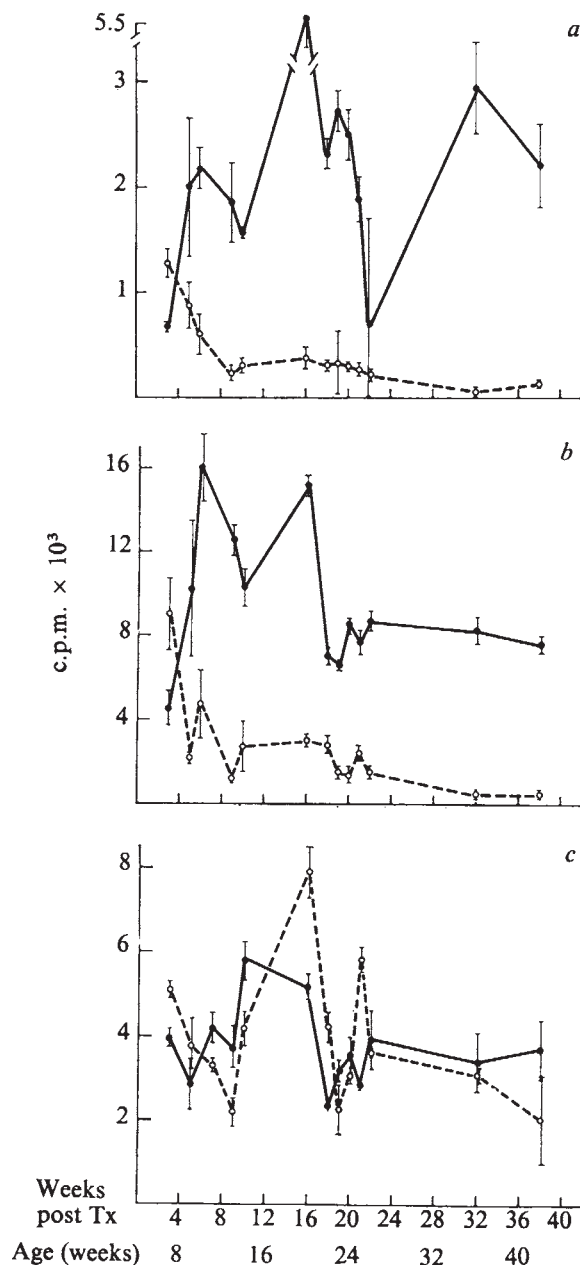


Fig. 1 Responses of 2.5×10^5 spleen cells from ATX (○) and STX (●) BDF₁ mice to (a) PHA, (b) con A, and (c) LPS at indicated times after thymectomy. All 26 pools of spleen cells were cultured simultaneously with the various mitogens. Female BDF₁ (C57BL/6 female $\times$ DBA/2 male) mice bred at the University of California, San Diego, or purchased from Jackson Laboratories were used in all experiments. They were subjected to complete thymectomy (TX) or sham-thymectomy at 4 weeks of age, under light Avertin anaesthesia according to the technique of Dischelar and Rudali¹⁶. On the day of the experiment animals were killed by cervical separation, the spleens removed aseptically and single cell suspensions prepared as described previously¹⁷. Counts and viability determination were obtained using a haemocytometer and trypan blue dye exclusion. The cells were then resuspended in RMP1-1640 (GIBCO) which was supplemented with 10% adult human serum from one of us (W.B.). 2.5×10^5 cells in 100 μ l were then added to individual

differences were not as striking. In contrast, the response to LPS, though erratic, was similar in both normal and ATX mice. The 50% depression in these animals 20–38 weeks after thymectomy was not a consistent finding; in some experiments ATX cells gave a higher response than STX cells in these older mice. Most investigators agree that this mitogenic response occurs in the absence of T cells. If any T-cell component is involved, it is too small to have been detected in these experiments.

We have explored the response of these animals further and examined the kinetics, mitogen dose-response, variation in the number of cells cultured, and the substitution of foetal bovine sera for adult human sera. In all cases the responses of ATX animals to PHA and con A were depressed between 8 and 26 weeks after thymectomy compared with STX controls.

The decay in mitogen responsiveness seen here is consistent with the existence of two populations of T cells—one required for the bulk of the response to mitogens which decays completely by 9 weeks after thymectomy, and another long lived, responding poorly to PHA and con A. Although there have been several reports that adult thymectomy has no effect¹⁰, or a transient effect^{11–13} on mitogen responsive cells, some investigators have observed a decline in certain T cells subsequent to the same procedure. Raff and Cantor⁷ and Kappler *et al.*⁸ have demonstrated a diminution of precursors of T helper cells within 6 weeks after adult thymectomy. Furthermore, the number of Thy 1.2 positive cells in the spleen decreases by 5 weeks after adult thymectomy, caused primarily by a loss of those cells with a high rather than a low density of that antigen⁹. Moreover, rapidly dividing T cells found primarily in the spleen of normal mice are more sensitive to the cytolytic activity of anti-Thy 1.2 (ref. 14) and are required for the mitogenic response to PHA¹⁵. These results have been attributed to the presence of a short lived splenic T cell responsible for PHA responsiveness and possessing a high density of Thy 1.2.

The time course of the response in ATX mice (Fig. 1) is not clearly separable into decay curves of two cell populations. It therefore could be argued that it represents the decay of one long lived cell activity. In this case mitogen responsiveness should not drop off so rapidly unless maintenance of existing T-cell activity is thymus-dependent. Removal of the thymus does, of course, prevent further maturation of cells in the thymus and periphery. To the extent that maturation results from peripheralisation of cells from the thymus, thymectomy would abolish emigration. A deficiency in continued maturation would suffice to explain the inability of ATX mice to achieve the high levels of PHA and con A responsiveness seen in controls between 10 and 20 weeks of age.

The lesion in mice thymectomised as young adults is seen here as an inability to reach mature levels of responsiveness to PHA and con A and a decline of existing reactivity to these mitogens by loss of either a responding cell or an

wells in microtitre plates (Cooke Engineering Co., Alexandria, Virginia) and a further 100 μ l added of either mitogen in serum-free medium or serum-free medium alone in the case of non-stimulated background cultures. Mitogens were used at the following final concentrations: 2.5 μ g ml⁻¹ PHA (Wellcome Research Laboratories, Beckenham, Kent), 10 μ g ml⁻¹ con A (Pharmacia), 20 μ g ml⁻¹ LPS (LPS W from *E. coli* 055:B5, Difco). The cultures were incubated at 37 °C for 44 h and then pulsed with 50 μ l of ¹²⁵I-5-iodo-2-deoxyuridine (¹²⁵IUDR) (1 μ Ci ml⁻¹, specific activity > 200 Ci mmol⁻¹, New England Nuclear Corp.) for a further 12 h. The cultures were then removed and collected using a slightly modified multiple cell culture harvester¹⁸ (Otto Hiller Corp., Madison, Wisconsin). The cells collected on to the filter paper were rinsed with saline, 15% trichloroacetic acid and 95% ethanol before being counted in a Nuclear Chicago automated gamma-counter. Cells cultured without added mitogen contained less than 115 c.p.m. Data given are mean c.p.m. of four cultures $\pm$ s.d.

amplifying cell. Our data do not permit a precise evaluation of the half life of this activity.

We thank Ms Mary Coleman for technical assistance. This work was supported by grants from the National Institute for Allergy and Infectious Diseases, the National Cancer Institute, the National Foundation, the Rockefeller Foundation, and the Cancer Coordinating Committee of the University of California, and a fellowship from the Cancer Research Institute, Inc.

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Received December 23, 1974; revised February 27, 1975.

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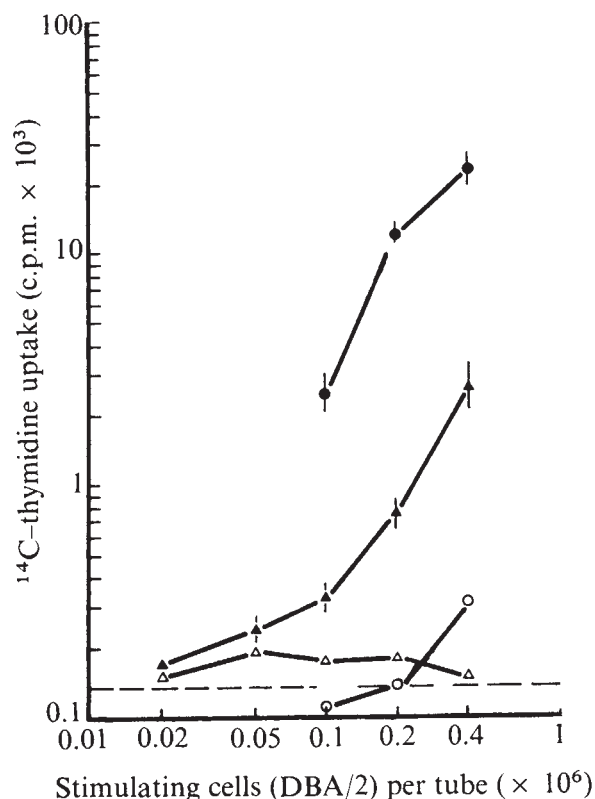


Fig. 1 Dose-response curves of mixed culture reactions with varying numbers of DBA/2 macrophages (●) or DBA/2 lymph node lymphocytes (▲) as stimulating cells and 0.6×10^6 BALB/c lymphocytes per tube as responding cells. Open symbols, respective stimulating cells alone. ^{14}C -thymidine uptake was measured on day 2-3. Dashed line indicates the uptake by the responding cells cultured alone.

Specific lymphocyte-activating determinants expressed on mouse macrophages

WHEN two populations of allogeneic lymphoid cells are cultured together, cellular interactions lead to blast transformation and proliferation of a portion of the cultured cells. In mice, this mixed leukocyte culture reaction (MLR) is under the independent control of two genetic systems: the multigenic major (H-2) histocompatibility system (MHS)^{1,2} and the unigenic (non-H-2) *M* locus³. The nature and cellular distribution of the gene products which mediate recognition of one cell (the stimulator cell) by another cell (the responder cell) are incompletely defined. The 'recognising' structure is likely to be a specific receptor present on thymus-derived (T) lymphocytes, since T cells are generally required as responder cells in MLR³⁻⁶. Recently it has been possible to distinguish, from these recognition sites, gene products which are expressed on the stimulator cells⁷ and which have been termed lymphocyte-activating determinants (LADs)³. Cell fractionation studies revealed that LADs of the MHS are expressed on both B and T lymphocytes^{8,9}, whereas LADs of the *M* locus are preferentially if not

exclusively expressed on B lymphocytes¹⁰. In this study we present evidence that LADs of the *M* locus as well as LADs of the MHS are also expressed on macrophages. These genetic determinants are shown to be stable in long term cultures and to induce specific one-way reactions in mixed lymphocyte-macrophage cultures.

Macrophage monolayers were prepared in flat-bottomed 9×44 mm polystyrene tubes using 4×10^5 macrophages obtained from the peritoneal cavity of normal mice (J. R.-M., V.S., F. Garrido and H.F., unpublished). After removal of non-adherent cells by several washings 6×10^5 lymphocytes obtained from lymph nodes of allogeneic mice were added in a total volume of 0.4 ml. These mixed cultures were incubated for 48 h at 37 °C in an atmosphere of 10% O_2 , 4% CO_2 and 86% N_2 . ^{14}C -thymidine (20 μl ; 60 mCi m Mol^{-1}) was then added and the tubes were incubated for a further 16 h. The non-adherent cells were collected onto a filter using a semi-automatic harvester and their isotope uptake measured in a liquid scintillation spectrometer.

Mouse lymphocytes cultured with allogeneic or semi-

Table 1 Proliferative response of mouse lymphocytes cultured with allogeneic or semiallogeneic macrophages

Incompatibility*	Stimulating macrophages	Responding lymphocytes	^{14}C -thymidine uptake†	Factor of stimulation‡
<i>M</i> locus	(BALB/c $\times$ DBA/2) F_1	BALB/c	$10,307 \pm 146$	9.1
	BALB/c	(BALB/c $\times$ DBA/2) F_1	$1,133 \pm 125$	1
H-2	(BALB/c $\times$ CBA/H) F_1	BALB/c	$5,601 \pm 416$	5.2
	BALB/c	(BALB/c $\times$ CBA/H) F_1	$1,086 \pm 246$	1
<i>M</i> locus	DBA/2	BALB/c	$12,453 \pm 435$	12.7
	BALB/c	DBA/2	978 ± 12	1

* BALB/c = H-2^d, Mls^b; DBA/2 = H-2^d, Mls^a; CBA/H = H-2^k, Mls^b.

† Mean c.p.m. $\pm$ s.d. from triplicates; uptake of the macrophages cultured alone was in the range 300-500 c.p.m.; uptake of the lymphocytes cultured alone was in the range 50-200 c.p.m. Uptake by F_1 lymphocytes cultured with parental macrophages or of DBA/2 lymphocytes cultured with BALB/c macrophages was not significantly higher than the uptake by syngeneic cultures (not shown).

‡ Comparing uptake obtained in one direction with the uptake obtained in the other direction.

Table 2 Proliferative response of mouse lymphocytes cultured with highly purified allogeneic or semiallogeneic macrophages

Stimulating macrophages*	Responding lymphocytes†				
	None	BALB/c	DBA/2	(BALB/c × DBA/2)F ₁	AKR/J
—		65	84	41	84
BALB/c	77	762 (1)	393 (1)	338 (0.3)	1,237 (3§)
DBA/2	146	13,943 (18‡)	279 (1)	303 (0.3)	1,440 (3.5§)
(BALB/c × DBA/2)F ₁	114	4,793 (6‡)	243 (1)	995 (1)	2,078 (5§)
AKR/J	90	7,786 (10)	1,099 (4§)	2,594 (3§)	404 (1)

* Peritoneal macrophages were precultured for 10 d. More than 95% of the monolayer cells stained for lysosomes with acridine orange. No lymphocytes or PMN could be detected. The monolayers were washed, incubated with 0.25% trypsin solution for 30 min at 37 °C, washed again and then used as stimulating cells.

† ¹⁴C-thymidine uptake in c.p.m. (mean of triplicates) obtained in the various cultures contained lymphocytes alone (first horizontal row), macrophages alone (first vertical row) or both together in all possible combinations. The numbers in brackets indicate the factor of stimulation based on the uptake observed in the syngeneic mixed cultures.

‡ Incompatibility at the *M* locus (unilateral stimulation by LAD of Mls^a); AKR/J = H-2^k, Mls^a; for other strains see Table 1.

§ Incompatibility at H-2.

|| Incompatibility at the *M* locus and at H-2.

allogeneic macrophages showed specific proliferative responses (Table 1). Parental lymphocytes responded well to F₁ macrophages differing either at the *M* locus or at the H-2 complex, but F₁ lymphocytes did not respond to parental macrophages. The one-way reaction in the lymphocyte-macrophage mixed cell culture system is further demonstrated by the results obtained with cells from the H-2^d identical mice DBA/2 and BALB/c. Unilateral stimulation of BALB/c by DBA/2 has been reported with lymphocytes as stimulator cells¹¹ and this has been attributed to the strong lymphocyte-activating determinant coded for by the *Mls^a* allele at the *M* locus¹². It therefore seems that LADs of the H-2 complex as well as LADs of the *M* locus are expressed on macrophages.

The possibility that stimulation in our mixed cultures was caused by contaminating lymphocytes or polymorphonuclear cells (PMN) was excluded in various ways: (1) pretreatment of the macrophage monolayers with anti-B cell and anti-T cell serum¹³ plus complement; (2) pretreatment of the monolayers with trypsin combined with extensive washing (J. P.-M., V.S., F. Garrido and H.F., unpublished); (3) preculturing the macrophages for about 10 d thus allowing PMN¹⁴ and probably also lymphocytes to die; and (4) combining methods (2) and (3). None of these procedures reduced the stimulating capacity of the macrophage monolayers. Table 2 shows an experiment in which macrophages obtained from 10 d cultures were treated with trypsin to detach any remaining lymphocytes and to remove dead cell material, and extensively washed before they were used as stimulator cells in mixed cultures. Specific proliferative responses were again observed when the cells were incompatible at the *M* locus, at the H-2 or both.

Interestingly, *M*-locus incompatibility between lymphocytes and macrophages resulted in quite strong stimulation, whereas H-2 incompatibility only led to comparatively weak stimulation. Figure 1 shows a dose-response curve of the stimulation obtained in an *M* locus incompatible combination (DBA/2 against BALB/c). DBA/2 macrophages seemed to have stronger stimulatory capacity than DBA/2 lymphocytes. Further experiments are in progress to determine the relative stimulatory capacity of macrophages compared with other non-lymphoid or lymphoid subpopulations of cells, and to establish the genetic mapping of the LADs expressed on macrophages by testing congenic and H-2 recombinant mice.

Studies on macrophage-lymphocyte interactions in MLR date back to the early days of the MLR. Although there is general agreement that macrophages are required in the reaction, reports on their specific contribution to the allogeneic stimulus have been conflicting. Some of the results are difficult to interpret because the numbers of monocytes or macrophages were not quantitated. Wherever human purified macrophages were tested directly, they were found to be powerful stimulators of allogeneic lymphocytes^{15,16}. Other non-lymphoid human cells known to carry histocompatibility antigens on their surface have usually not been found to stimulate allogeneic lymphocytes *in vitro*. In the guinea pig, macrophages were

recently found to be the predominant stimulator of the MLR, far more effective than lymphocytes, fibroblasts or PMN¹⁷. Mouse peritoneal macrophages were found to be immunogenic not only in MLR, as described here, but also when used for induction of cytotoxic cells¹⁸ or cells exhibiting the graft-versus-host reaction¹⁹.

The importance of the macrophage for the immune system has recently been firmly established. Many observations suggest a function for the macrophage in antigen presentation. This may explain their requirement, for example, in soluble antigen-mediated T lymphocyte proliferation²⁰, MLR²¹, induction of helper T lymphocytes²² and cytotoxic effector cells²³, and in T-B cell cooperation *in vitro* leading to antibody synthesis²⁴. Macrophages have also been found to be involved in the genetic control of certain immune responses²⁵. Cooperation between macrophages and T cells was found to depend on a certain compatibility, which was associated with the I region, possibly with Ia antigens, of the H-2 complex (P. Erb, personal communication). Clearly, the study of macrophage cell-surface components, like Ia antigens and LADs will become increasingly important for the understanding of the nature of macrophage-lymphocyte interactions.

This work was supported in part by the Cancer Research Campaign and the Wellcome Trust. We thank Mrs Barbara Reynolds for assistance.

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Control of flagellar wave movement in *Crithidia oncopelti*

MECHANISMS controlling cell behaviour are of considerable importance in biology and medicine, and much effort has been expended in experiments designed to evaluate the biochemical events which are involved in the mediation of a variety of cellular activities. Calcium ions are found to be involved in the regulation of a number of motile processes, such as muscular contraction¹, ciliary reversal² and cytoplasmic contraction³. We present here preliminary results which show that calcium may be responsible for the control of flagellar activity in a trypanosomid flagellate, *Crithidia oncopelti*. The flagellum of this organism propagates waves both distally and proximally in common with other trypanosomes⁴ but in contrast to the flagella on the majority of other protozoa and spermatozoa which are observed to maintain only distally propagating waves⁵. During normal swimming *Crithidia* propagates waves from tip to base, but on meeting an obstruction wave reversal occurs for several flagellar beats, thereby causing the organism to reverse its direction of movement and enabling it to escape from the obstacle. The wave reversal thus seems to be an avoidance reaction, and can be induced by mechanical, electrical or chemical stimulation⁶.

Cells of *Crithidia* have been extracted in glycerol⁷ and detergent-based⁸ solutions in such a way that the addition of ATP and magnesium to the preparation induces wave motion. The character of the reactivated flagellar movements is critically dependent on a number of physical and chemical factors during extraction and reactivation; with the detergent-based technique higher flagellar beat frequencies are obtained than for corresponding conditions with the glycerol-based method. In a previous report on the behaviour of glycerol-extracted cells⁷ the wave direction was from the basal end of the flagellum only, which seemed to have lost the ability to reverse the direction of wave propagation. In those experiments, however, no attempt was made to control the concentration of calcium ions, which we have now found to have a significant effect on the behaviour of the system.

In our experiments the reactivation solutions contained ATP and magnesium at a concentration of 1 mol m^{-3} (1 mM) and the concentration of free calcium in the solutions was controlled by the addition of EGTA. The amount of calcium in the solutions was determined using an atomic absorption spectrometer, and the concentration of free calcium ions was calculated using a method described previously⁸. Unless the calcium concentration was low in both the extraction and reactivation solutions, waves are propagated from the base of the flagellum only. When the concentration of calcium is maintained below about $10^{-4} \text{ mol m}^{-3}$ (10^{-7} M), waves propagate from tip to base immediately after reactivation, but reverse after a time, which depends on the preparative technique. Using a procedure based on glycerol, tip-to-base propagation was observed for about 20 min and occasional reversal was seen during this time. Extraction and reactivation with detergent-based solutions produced waves which propagated from the tip for a period of between 2 and 4 min; no reversal was observed during this time. Electron microscopy shows that the glycerol-extracted preparations have fragments of membrane associated with the axonemal structure whereas the cells extracted with detergent have no visible membrane.

The effect of calcium ions on flagellar activity has also been investigated using ionophores, X537A and A23187, known to transport divalent ions across membranes⁹. With cells *in vivo*, the presence of either of the ionophores and $1\text{--}5 \text{ mol m}^{-3}$ ($1\text{--}5 \text{ mM}$) CaCl_2 caused the majority of organisms in a sample to propagate waves from base-to-tip—that is, in the opposite direction to that normally expected of the cells *in vivo*. When EGTA was added to the medium instead of CaCl_2 a few

organisms propagated waves from base-to-tip but the majority maintained proximally directed waves. When ionophore, CaCl_2 or EGTA were added separately to a suspension of the organisms, in the concentrations used during the experiments, they caused no abnormal flagellar movements.

These results suggest strongly that calcium ions are able to control the direction of flagellar wave propagation in the preparations of *Crithidia* used. Note that the experiments described here were performed on *in vitro* systems and that the control mechanism may be different in the living cell. Nevertheless many features of the motility of the systems used are similar to those of the living organism and it is tempting to speculate that adjustment of the calcium concentration in the living cell is used to alter the direction of propagation of the flagellar waves.

The behaviour of the systems studied favours the idea that the membrane is intimately involved in the control mechanism in *Crithidia*, as suggested previously⁴. In those preparations in which the membrane is completely dissolved, namely the detergent-extracted systems, proximally directed wave propagation occurs for a few moments only after reactivation. The other systems studied are known to have residual membrane structures, although for the glycerol-extracted preparation the membrane is considerably disrupted, and show wave propagation from tip-to-base for considerable periods of time, with occasional wave reversal. A possible interpretation of these results is that in a living cell the membrane acts as a receptor for extracellular events and regulates the intra-flagellar calcium concentration, which, in turn, controls the reaction determining the direction in which waves will be propagated. An alternative explanation of the difference in behaviour between the detergent- and glycerol-extracted cells is that, in the latter case, preservation of the complete motile system is sustained for a longer period. It is well known that glycerol acts as a preservative for spermatozoa with similar axonemal structures to that of *Crithidia*, whereas detergents solubilise protein structures.

The site of calcium activity and sequestration within the flagellum is a matter for speculation at present, but a reasonable suggestion can be made with reference to the currently favoured sliding filament hypothesis¹⁰ of flagellar activity, according to which the forces required to bend a flagellum originate in active relative sliding of the outer doublet microtubules of the axoneme. The ability to alter the direction of wave propagation in *Crithidia* implies that, for this organism at least, the relative direction of active filament sliding can be reversed. If the sliding is induced by swinging movements of linkages between the outer tubules of the flagellar axoneme it is possible that the effect of calcium is to regulate the direction in which a link will swing. If this is the case the close association of the membrane and the outer axonemal tubules would enable a stimulus originating at the membrane to be transferred quickly to the site of active force development in the organelle. It is possible that the flagellar membrane acts as the storage site for calcium ions, so that the appropriate stimulus could cause the release or sequestration of ions by the membrane, thus causing the appropriate reversal of flagellar bend movement.

Calcium has been found to have an effect on the behaviour of two other systems which carry organelles with the same 9+2 axonemal structure of the *Crithidia* flagellum. In chemically extracted *Paramecium*, ciliary reversal, which is an avoiding response *in vivo*, can be induced by raising the concentration of external calcium²; these observations support Naitoh's¹¹ view that ciliary activity in the organism is controlled by two physiological systems each of which utilises ATP. One system, activated by magnesium, is responsible for bending the cilia, whereas the other, calcium-activated, determines the direction of beating. The mechanisms of ciliary reversal could be the same as that of wave reversal in *Crithidia*, since the two activities may result from a changed sliding direction of the outer tubules. Thus, like *Paramecium*, *Crithidia* may have separate systems for propagating bends and for initiating the avoiding response, both

deriving energy from ATP hydrolysis, with the former activated by magnesium and the latter by calcium.

The other system referred to is the sea urchin spermatozoa, the flagellar waveforms of which can be altered by varying the calcium concentration¹². We have found that in addition to controlling the direction of flagellar beating, variations in the calcium concentration cause modifications in the wave shape of the flagellum of *Crithidia*. This may bear a relationship to the behaviour of sea urchin spermatozoa but we have insufficient information at present to make a detailed comparison between the two. Further experiments are in hand to quantify the effects of calcium on motility and to locate the sites of activity and sequestration of this ion within the flagellum.

We thank Roche Products Ltd, and Lilly Research Centre Ltd, for respective gifts of ionophores, X537A and A23187. This work was supported in part by a grant from the SRC. We also thank Dr M. Hughes for discussion and for assistance with the operation of the atomic absorption spectrometer.

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Received February 21; accepted March 27, 1975.

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Comparison of doubling numbers attained by cultured animal cells with life span of species

HUMAN fibroblasts in culture undergo 40-60 cell doublings (indicating numbers of cell generations) before the growth rate declines and death of cells ensues¹. Hayflick² has suggested that the final doubling numbers achieved by cultured diploid cells may be related to the life span of the animal species from which the cells were derived. This suggestion

is based on reported final doubling numbers for human, chicken and mouse being in the ranges 40-60, 15-35, and 14-28, whereas the maximum life span of each of these species was 110, 30 and 3.5 yr, respectively. We report here findings on final cell doubling numbers attained when cells from ten different animal species were serially subcultured. Tissues of foetal or newborn origin were used to initiate cultures from man, horse, monkey, cat and dunnart, and the cultures of wallaroo×red kangaroo hybrid, kangaroo, wallaby, rabbit and potoroo, were derived from adult tissues.

After reaching appropriate cell doubling numbers (Table 1) cell cultures derived from man, horse, wallaroo×kangaroo hybrid, kangaroo, wallaby and potoroo showed a decline in growth rate and an increase in cell granularity that could be compared with the characteristics described by Hayflick¹ as Phase III in the growth of human diploid cell strains. There was no evidence that technical procedures or infection with mycoplasmas or viruses caused this decline and final death of cultures. In the case of monkey, cat and rabbit, the cells continued to grow after the doubling numbers listed in Table 1 but counts after this time showed that there had been a shift from a diploid to a heteroploid chromosome constitution. There was no change in the growth rate or morphology of these cells at this time. On last examination, the cultures from monkey, cat and rabbit had reached 100, 100 and 70 cell doublings respectively. On the other hand, the dunnart culture when last examined after 170 cell doublings had shown no change in growth rate and no change from the diploid chromosome pattern.

In our studies, the final cell doubling number for their diploid cells in the species listed cannot be related to their life spans. This applies to those which remained diploid throughout their lifetime and to those which subsequently became heteroploid. Note that these doubling numbers are based on only one culture series for each species. The final cell doubling number attained by each species may well be found to be higher when cells are cultured from a large number of individuals within one species, as experience with diploid cells cultured from a large number of human embryos has shown the final doubling number to be in the range 40-60. Adult tissues were used to initiate five of the cultures. In extensive studies on human tissues ranging in age from foetal to 90 yr, Martin *et al.*⁷ showed that final doubling numbers decreased by 0.2 population doublings per year of donor life. We may therefore have obtained higher final doubling numbers for wallaroo×red kangaroo hybrid, kangaroo, wallaby, rabbit and potoroo if foetal tissue had been used, thus making the association between life

Table 1 Comparison of doubling numbers

Species	Maximum lifespan (yr)	No. of doublings attained by diploid cells	Tissue of origin of cells
Human, <i>Homo sapiens</i>	110 ⁴	60	Lung
Horse, <i>Equus caballus</i>	46 ⁵	82	Kidney
Monkey, <i>Macaca speciosa</i>	34 ⁵	60	Lung
Cat, <i>Felis cattus</i>	28 ⁵	92	Lung
Wallaroo, <i>Macropus robustus robustus</i>	19 ⁵	65	Skin*
×			
Red kangaroo hybrid, <i>Megaleia rufa</i> Hybrid			
Grey kangaroo, <i>Macropus gigantus</i>	16 ⁵	46	Skin*
Pretty-faced wallaby, <i>Macropus parryi</i>	16 ⁵	46	Heart*
Rabbit, <i>Oryctolagus cuniculus</i>	13 ⁵	70	Skin*
Potoroo, <i>Potorous tridactylus</i>	7 ⁵	40	Heart*
Fat-tailed dunnart, <i>Sminthopsis crassicaudata</i>	3 ⁶	170	Skin

* Cell lines derived from adult tissue. Other lines derived from foetal or newborn tissue. Cultures were initiated from small pieces of tissue from various sites. Growth medium was Basal Medium Eagle's (BME)² plus 10% foetal calf serum. Cultures from all species showed a fibroblast-like morphology, which persisted throughout the experiments, and were subcultured when confluent (within the range 4-7 d). Most cultures were divided 1:4 at subculture. Rabbit and dunnart cells grew more rapidly and could be divided 1:8 or 1:16, whereas the cultures derived from kangaroo grew more slowly and were divided 1:2. Chromosome examinations were made at regular intervals on all cell cultures to ensure that the normal diploid number of chromosomes was present.

spans of species and the final doubling numbers of their cultured cells more obscure.

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Received December 3, 1974; accepted March 27, 1975.

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Heat-labile enzymes in Werner's syndrome fibroblasts

RECENT reports indicate^{1,2} that cultured skin fibroblasts from subjects with Werner's syndrome, a hereditary disease of premature ageing³, have an increased proportion of heat-labile glucose-6 phosphate dehydrogenase (G-6PD) compared with normal cells undergoing ageing *in vitro*^{1,2,4}. Additionally, two HL-A antigens show profoundly altered reactivities with specific antisera², which in total demonstrates that three diverse gene products are markedly abnormal in Werner's fibroblasts. The purpose of our study was to document further this phenomenon in Werner's cells. We now report a high heat-labile fraction of 6-phosphogluconate dehydrogenase (6-PGD), an enzyme involved in the hexose monophosphate shunt, and hypoxanthine-guanine phosphoribosyltransferase (HGPRT), an enzyme concerned with purine metabolism.

Table 1 Heat-labile fraction of enzymes in cultured fibroblasts from a subject with Werner's syndrome and controls

	% Heat-labile enzyme 6-Phosphogluconate dehydrogenase	Hypoxanthine- guanine phosphoribo- syltransferase
Werner's syndrome		
Experiment 1	14	50
Experiment 2	24	36
Experiment 3	—	40
Controls		
Early passage	0.80 ± 0.43 (n = 10, range 0-4)	7.66 ± 1.70 (n = 9, range 0-14)
Late passage	4.89 ± 1.16 (n = 12, range 0-9)	24.43 ± 2.84 (n = 7, range 15-32)

Werner's cells were within 3-6 mean population doublings (MPD) of termination in a total replicative life span of 18 MPD; the limited number of experiments was dictated by the poor growth of these cells and finally exhaustion of their supply. Control values (mean ± s.e.m.) represent experiments on strains of cultured cells from five normal donors aged 27-76 yr. Early passage cells were in the first third of their replicative life spans whereas late passage cells were within 0-9 MPD of growth termination (range of life spans 44-70 MPD). Werner's values are significantly different from late passage controls, $P < 0.01$. Controls at late passage were significantly different from early passage controls, $P < 0.01$.

Cultures were derived and propagated as described previously². The assays for 6-PGD and HGPRT and the estimates of heat lability, which were accurate within 3-4%, were carried out as before^{4,7}. Control strains were studied at early and late passage, the latter serving as more suitable controls for the poorly growing Werner's cells.

Table 1 indicates that normal fibroblasts accumulated an increased fraction of heat-labile 6-PGD and HGPRT during the culture life span. But Werner's cells clearly contained a higher proportion of heat-labile enzyme, even when compared to control fibroblasts at late passage. Mixing experiments with

equal parts of crude extracts from Werner's and control strains gave approximately intermediate heat-labile fractions suggesting that neither proteolysis nor a deficiency of stabilising factors was responsible for the results observed in Werner's cells^{1,2,4,7}. The high heat-labile fraction of HGPRT in late-passage controls considerably exceeds that of 6-PGD and previous data on G-6PD^{1,2} for reasons that are uncertain but which probably relate to loss of the stabilising cofactor during the dialysis performed before HGPRT assay⁷.

The data indicate that 6-PGD, an enzyme coded for by an autosomal gene and the X-linked HGPRT⁷ are markedly abnormal in Werner's fibroblasts at the same time as alterations appear in G-6PD and HL-A antigens, genetic loci that are also X-linked and autosomal, respectively^{6,7}. The results also show an increasingly defective fraction of 6-PGD and HGPRT in normal cells during ageing *in vitro*, as was found earlier for 6-PGD and G-6PD^{1,2,4,7,8}. Indeed, similar abnormalities of the identical gene products have now been demonstrated in cultured cells derived from subjects with progeria, a related disorder of premature ageing⁵⁻⁸. The molecular nature of these defects is unclear and may even be unique in each case^{7,8}. It is also not certain whether defective proteins are the consequence rather than the cause of cellular ageing. But these observations taken together with the decreased replicative potential that occurs during normal ageing^{6,9,10} and the more severe growth limitation observed in Werner's and progeria fibroblasts^{6,10}, strengthen the view that the rate of fibroblast ageing *in vitro* is directly related to the rate of ageing *in vivo*^{1,2,4-10}.

This work was supported by grants from the Medical Research Council of Canada and the Canadian Diabetic Association Foundation Fund during the tenure of a scholarship from the MRC (to S.G.).

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Received January 24; accepted April 4, 1975.

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Morphine abstinence is associated with increased brain cyclic AMP

THE finding that theophylline, which inhibits brain cyclic AMP phosphodiesterase¹, produces a "quasi-morphine abstinence syndrome" that is intensified by naloxone and suppressed by heroin² suggested that the real morphine abstinence syndrome may be associated with increased activity of a brain cyclic nucleotide. A suitable phosphodiesterase inhibitor would therefore heighten and a stimulant of phosphodiesterase would lower the intensity of real morphine abstinence signs. To test this, we administered such drugs to morphine-dependent rats shortly before withdrawal was precipitated with naloxone, so that the drugs would act only during the expression of abstinence. As these experiments indicated that the abstinence syndrome is largely associated with increased cyclic nucleotide activity, we attempted to identify the nucleotide by intracerebroventricular injection of cyclic nucleotides. These experiments indicated that cyclic AMP rather than cyclic GMP was primarily involved.

Opiate dependence was induced in male white Wistar rats (100-150 g) by a single subcutaneous injection of morphine, 150 mg kg⁻¹, in a sustained-release preparation³. Withdrawal was precipitated 24 h later with 0.1 or 1 mg kg⁻¹ subcutaneous

(s.c.) naloxone hydrochloride in 0.9% w/v saline. To modify withdrawal effects, imidazole (which stimulates phosphodiesterase⁴, including that of rat brain⁵), theophylline or caffeine¹ was given orally in water 2 h before naloxone challenge; or 3-isobutyl-1-methylxanthine (IBMX), which inhibits cyclic AMP phosphodiesterase of rat brain (A. C. Roy, personal communication), guinea pig brain⁶ and mouse neuroblastoma cells⁷, was given subcutaneously in saline, 1 h before challenge. Alternately, cyclic AMP, cyclic GMP or the dibutyl derivative of each, dissolved in sterile saline, was injected into the left lateral cerebral ventricle of the brain through an indwelling cannula⁸, 0.5 or 1 h before challenge. After challenge with naloxone or saline, behavioural responses were recorded by observers who did not know what treatment each animal had received. Because the incidence of abstinence effects is related to the cage in which the animal is situated, rats were observed individually, either in plastic buckets, in which jumping was the predominant abstinence effect; or, for other behavioural responses, in low cages that suppressed jumping. Jumps were counted, and other withdrawal signs were recorded as present or absent. The significance of differences between jumping scores, and between total scores for the abstinence syndrome as a whole, were tested by the Mann-Whitney *U* test⁹, adapted for the computer¹⁰. For quantal counts, Fisher's Exact Probability Test⁹ was used.

As jumping was a characteristic and readily quantifiable

sign of opiate abstinence, we tested how far drugs that modify brain phosphodiesterase activity affected the jumping of dependent rats when abstinence was precipitated with the higher dose of naloxone (1.0 mg kg⁻¹). Table 1 shows that much jumping occurred in rats that had received sustained-release morphine, followed by a phosphodiesterase inhibitor and naloxone. When either morphine or phosphodiesterase inhibitor was omitted from the treatment schedule, jumping was markedly reduced. Imidazole, in dependent rats, significantly decreased jumping and overcame the ability of theophylline to increase jumping.

These results suggested that this expression of abstinence was associated with an increase in brain cyclic nucleotide. To identify the nucleotide, we administered cyclic AMP, cyclic GMP or one of their dibutyl derivatives into the left lateral cerebral ventricle of morphine-dependent rats and observed jumping after precipitating abstinence with naloxone (1.0 mg kg⁻¹). Intracerebroventricular injection of cyclic AMP and, to a lesser extent, dibutyl cyclic AMP significantly increased jumping (Table 1). Cyclic GMP did not do so, although dibutyl cyclic GMP increased jumping to an extent that approached statistical significance (*P* = 0.052). Dibutyl cyclic GMP, however, significantly decreased two abstinence signs, teeth chattering (*P* < 0.05) and chewing (*P* < 0.01). That these were the two signs that IBMX also significantly lessened at 5–7 min after challenge (see below) suggested that

Table 1 Effects of cyclic nucleotides or of drugs inhibiting or stimulating phosphodiesterase on naloxone-precipitated jumping in morphine-dependent rats

Treatment			No. of rats	Rats jumping (%)	Jumping responses	
0 h	22 h	23 h			Mean no. of jumps	Median no. of jumps
Vehicle	Th, 100	—	10	10	0.4	0
Mo, 150	Vehicle	—	39	87	7.4	4.0
Mo, 150	Th, 100	—	38	100*	60.5	49.0‡
Mo, 150	Vehicle	—	11	82	4.9	4.0
Mo, 150	Th, 25	—	10	100	17.7	14.5*
Mo, 150	Th, 50	—	11	100	36.6	33.0†
Mo, 150	Vehicle	—	10	80	3.5	2.0
Mo, 150	Cf, 25	—	10	100	10.1	3.5
Mo, 150	Cf, 50	—	11	100	10.4	12.0†
Mo, 150	Cf, 100	—	10	100	22.6	12.0‡
Vehicle	—	IBMX, 10	10	30	2.3	0
Mo, 150	—	Vehicle	8	63	3.6	2.0
Mo, 150	—	IBMX, 2.5	8	88	11.6	5.5
Mo, 150	—	IBMX, 5.0	8	88	15.4	8.5
Mo, 150	—	IBMX, 10.0	8	100¶	44.0	27.5‡
Mo, 150	Vehicle	—	10	100	8.8	6.5
Mo, 150	Im, 250	—	10	60	3.3	1.5*
Mo, 150	Th, 100	—	10	100	63.0	46.5
Mo, 150	Th, 100 + Im, 250	—	11	100	19.9	11.0†
Mo, 150	—	Vehicle	16	50	1.75	0.5
Mo, 150	—	cAMP, 50	18	83*	8.17	4.0†
Mo, 150	—	Vehicle	11	87	3.5	1.0
Mo, 150	—	dbcAMP, 25	13	92	8.5	8.0*
Mo, 150	—	Vehicle	36	58	2.9	1.0
Mo, 150	—	dbcAMP, 50	34	61	5.6	1.0
Mo, 150	—	Vehicle	25	52	4.1	1.0
Mo, 150	—	cGMP, 50	26	69	4.1	3.0
Mo, 150	—	Vehicle	34	50	3.6	0.5
Mo, 150	—	dbcGMP, 50	36	67	5.0	2.0

At 24 h every rat received naloxone (1.0 mg kg⁻¹ s.c.) in saline. Values for jumping responses are based on the count for 15 min immediately after naloxone challenge.

Mo, sustained-release subcutaneous morphine; Th, theophylline given orally in water; Cf, caffeine given orally in water; IBMX, 3-isobutyl-1-methylxanthine subcutaneously in saline; Im, imidazole given orally in water; —, no treatment. Oral or subcutaneous drug doses in mg per kg of base. cAMP, cyclic AMP; dbcAMP, dibutyl cyclic AMP; cGMP, cyclic GMP; dbcGMP, dibutyl cyclic GMP; given through an indwelling polyethylene cannula into the left lateral cerebral ventricle. Intracerebroventricular drug doses in µg per rat.

P values for the jumping scores were calculated by the Mann-Whitney *U* Test. For comparisons of test drug with vehicle in morphine-dependent rats: **P* < 0.05, †*P* < 0.01, ‡*P* < 0.001. For comparisons of test drug in rats treated or not treated with morphine, §*P* < 0.05, ¶*P* < 0.01, ||*P* < 0.001.

Table 2 Intensification by IBMX or cyclic AMP of the abstinence syndrome in morphine-dependent rats

Response	% Incidence of responses after treatment schedule				
	Experiment with IBMX			Experiments with cyclic AMP	
	(a) V+I+N	(b) M+V+N	(c) M+I+N	(d) M+V+N	(e) M+A+N
Jumping	NT	NT	NT	NT	NT
Teeth chattering	0	0	6.7	100	87
Irritable to touch	60	67	100 ^{ab}	43	74 ^d
Irritable to handling	33	47	100 ^{ab}	67	78
Diarrhoea	0	40 ^a	53 ^a	52	65
Chewing	33	33	33	90	87
Ptosis	53	53	73	24	52
Body shakes	0	0	6.7	38	61
Head shakes	0	0	0	10	48 ^d
Paw tremor	0	0	27 ^{ab}	10	22
Rearing	0	13	60 ^{ab}	100	100
Restlessness	6.7	0	40 ^{ab}	62	78
Salivation	0	0	27 ^{ab}	0	0
Ejaculation	NT	NT	NT	43	78 ^d
Median total score*	2	3	5 ^{ab}	6	8 ^d

All rats were treated subcutaneously with 150 mg kg⁻¹ morphine (M) in a sustained-release preparation, or with vehicle (V) and, 24 h later, all were challenged with naloxone (N; 0.1 mg kg⁻¹ subcutaneously). In the experiment with IBMX, three groups, each of 15 rats, received 1 h before challenge, either IBMX (I; 10 mg kg⁻¹ s.c.) or vehicle, and responses were recorded during 15–17 min after challenge. In the experiments with cyclic nucleotides, groups totalling 21 and 23 rats received respectively into the left lateral cerebral ventricle, 0.5 h before challenge, either saline (V) or 50 µg cyclic AMP (A) and responses were recorded during 15 min after challenge.

Letters a–e in the body of the table indicate comparisons between columns where a significantly greater incidence was found; where the letter is italic, $P < 0.01$; where it is not, $P < 0.05$.

*The significance of the difference between intensities of the abstinence syndrome as a whole was based on the median total scores.

NT, Not tested or not recorded.

some increase in cyclic GMP, induced by inhibition of phosphodiesterase, may be part of the pattern of the quasi-abstinence syndrome, but that cyclic GMP contributes little to the real abstinence syndrome, in which both of these signs are prominent (Table 2, column d).

Certain drugs, such as atropine, which interfere with neurotransmitters inhibit some abstinence signs in the rat, but intensify others, whereas heroin lessens the incidence of all signs³. To determine how closely the substances under investigation were related to the generation of the abstinence syndrome as a whole, we studied the extent these substances affected other abstinence signs, as well as jumping. Three groups, each of 15 rats, received either sustained-release morphine or vehicle; 23 h later each group received IBMX or vehicle; and at 24 h, all were challenged with the lower dose of naloxone (0.1 mg kg⁻¹), and the presence of abstinence signs was recorded during two 2 min observation periods. When responses were recorded 5–7 min after challenge, irritability to touch ($P < 0.05$) and handling ($P < 0.001$), rearing ($P < 0.001$) and restlessness ($P < 0.001$) were significantly increased by IBMX; but teeth chattering ($P < 0.05$) and chewing ($P < 0.025$) were significantly reduced. At 15–17 min after challenge, however, six of the twelve individual abstinence signs were significantly increased by IBMX (Table 2, columns b and c). IBMX also increased the intensity of the abstinence syndrome as a whole ($P < 0.002$).

In two experiments, to test whether cyclic AMP increased other abstinence signs, as well as jumping, rats received sustained-release morphine and 23.5 h later, an intracerebroventricular injection of either 50 µg cyclic AMP or saline. Half an hour later, all rats were challenged with 0.1 mg kg⁻¹ naloxone and the occurrence of behavioural responses, other than jumping, was recorded. The incidence of body shakes ($P < 0.05$) and head shakes ($P < 0.025$) was significantly greater after cyclic AMP (ten rats) than after saline (nine rats). In a second experiment, the incidence of ptosis ($P < 0.025$) and of ejaculation ($P < 0.05$) was significantly greater in the cyclic AMP group (13) than in the saline group (12). None of the responses was significantly greater in the saline controls in either experiment. Table 2 (columns d and e) gives the pooled results of these two experiments, in which irritability to touch was also significantly greater in the rats receiving cyclic AMP. In these rats, also, the intensity of the abstinence syndrome as a whole was significantly greater ($P < 0.001$). In the first of these two experiments, a third group (nine rats) showed that intracerebroventricular cyclic GMP was no more effective than saline. After cyclic AMP, the abstinence syndrome as a whole

was more intense ($P < 0.05$), and the incidence of body shakes ($P < 0.005$) and of ejaculation ($P < 0.05$) was greater, than after cyclic GMP.

As inhibitors of phosphodiesterase intensified abstinence effects, we tested whether naloxone inhibited this enzyme. At concentrations up to mmol l⁻¹, however, naloxone did not inhibit cyclic AMP phosphodiesterase in rat brain homogenate (A. C. Roy, personal communication).

Our experiments show that drugs modifying the activity of brain cyclic AMP phosphodiesterase largely modify inversely the intensity of morphine abstinence effects. Thus the intensity of such effects can probably be associated with the level of cyclic nucleotide in brain cells. Injection of cyclic AMP intracerebroventricularly intensified several abstinence signs, without significantly diminishing any, whereas cyclic GMP reduced some of these signs, without significantly increasing any. Moreover, cyclic AMP intensified the abstinence syndrome as a whole, but cyclic GMP did not. These results argue that increase in cyclic AMP in appropriate brain neurones is associated with the generation of the morphine abstinence syndrome.

Reports on the relationship of abstinence effects to cyclic AMP in precipitated opiate withdrawal are consistent with our findings. Thus, a direct association was observed between whole brain cyclic AMP content and the intensity of the precipitated abstinence syndrome in morphine-dependent rats¹¹. Also, cyclic AMP, administered to dependent mice, intensified the weight loss, although not the jumping, of precipitated abstinence¹². These observations, coupled with our experimental findings suggest that opiate dependence is a state of potential increase in activity of a neuronal cyclic AMP mechanism, held in check by the presence of opiate, which inhibits an adenyl cyclase of morphine-sensitive neurones^{13–15}.

We thank Dr W. A. Klee, Mr A. C. Roy, Dr S. A. Saeed and Mr C. Schneider for helpful discussions; Mr M. A. Collins, Mr N. J. Cuthbert, Miss J. F. de C. Sutherland, Miss L. A. Taylor and Mr C. M. Tobin for observing animals, Mr L. C. Dinneen for statistical advice and Sankyo Limited for naloxone.

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Received December 16, 1974; accepted April 4, 1975.

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Impulse-flow regulation of high affinity choline uptake in brain cholinergic nerve terminals

ACETYLCHOLINE (ACh) synthesis is coupled, in some unknown manner, to ACh release caused by neuronal impulse flow¹⁻⁴. Accordingly, several models have been proposed regarding the regulation of ACh synthesis. These include feedback competitive inhibition of choline acetyltransferase by ACh^{5,6}, and mass action regulation of choline acetyltransferase⁷. More recently, high affinity choline uptake, which is very localised to cholinergic nerve terminals⁸, has been proposed as a regulatory step in ACh synthesis⁹⁻¹². If high affinity choline uptake were coupled to ACh synthesis and release, then one would expect changes in the high affinity choline uptake system after alterations in synthesis and release. We have found changes in the high affinity choline uptake system in hippocampal synaptosomes after treatments which alter neuronal impulse flow to cholinergic nerve terminals.

The rat hippocampus was used because of its known cholinergic innervation from the septum⁸, and because of the possibility of altering neuronal impulse flow in this tract^{4,13}. We used two means of slowing and/or stopping impulse flow. These were intraperitoneal injection of pentobarbital, and placement of acute radio frequency lesions in the septum under ether anaesthesia^{13,14}. The evidence that pentobarbital stops or slows impulse flow has been summarised and supported recently¹³. Placement of acute lesions would stop impulse flow by interrupting the axons. In pentobarbital-treated animals we have reversed the cessation of impulse flow by electrical stimulation of the medial septal area⁴.

A kinetic analysis of the high affinity choline uptake into hippocampal synaptosomes from untreated control animals revealed K_m and V_{max} values of about $0.8 \mu M$ and $60 \text{ pmol per 4 min per mg protein}$ respectively (Fig. 1, Table 1). These values are similar to those obtained by other workers^{9-11,15}. A similar analysis using synaptosomes from animals pretreated with pentobarbital (30 min) or septal lesions (1 h) revealed a decreased V_{max} (40%) with no change in the apparent K_m . When impulse flow to the hippocampus was restored in pentobarbital-treated animals by stimulating the septum for 10 min, the pentobarbital-induced reduction in V_{max} was reversed and the kinetic parameters returned to untreated control values (Fig. 1, Table 1). These kinetic changes were observed in every experiment (range of reduction in V_{max} in individual experiments was 30-65%). Complementary to the above changes, we have found that administration of scopolamine, an anticholinergic drug which would increase ACh turnover, and pentylenetetrazol, a central nervous system stimulant, results in a 25-50% increase in choline uptake.

No inhibition of uptake into synaptosomes from untreated animals was observed when $10^{-3} M$ pentobarbital

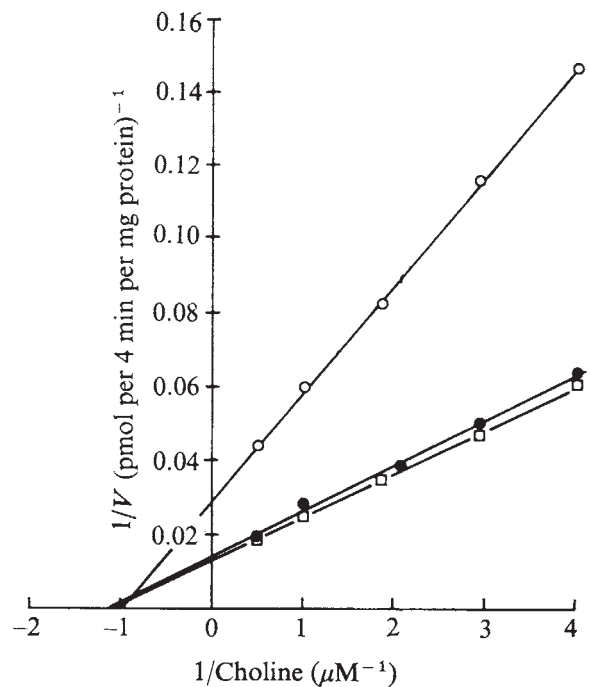


Fig. 1 Lineweaver-Burke analysis of high affinity choline uptake into hippocampal synaptosomes from untreated control rats, pentobarbital (65 mg kg^{-1}) treated rats, and electrically stimulated pentobarbital-treated rats. Male Sprague-Dawley rats (175-225 g) were administered pentobarbital intraperitoneally and decapitated 30 min later. Electrical stimulation⁴ (40 Hz, 200 μA) was begun 20 min after injection, and continued for 10 min. Animals were decapitated 7-10 s later. In all experiments, the hippocampus (whole, bilateral hippocampal formations) was dissected rapidly and homogenised in 20 volumes of 0.32 M sucrose. Crude mitochondrial fractions were isolated by differential centrifugation and resuspended in the original volume of 0.32 M sucrose. Samples (100 μl) of this suspension were added to 0.9 ml of Krebs-Ringer solution, and incubated at $30^\circ C$ for 4 min for uptake measurements^{12,14}. Blank values were obtained by maintaining parallel samples at $0-2^\circ C$. Choline concentrations added to the incubation media varied from 0.2 to $2 \mu M$. Higher concentrations were not used because uptake by the low affinity component would tend to predominate¹⁵. Lines were fitted to the data points by least squares analysis. $\circ$, Pentobarbital; $\bullet$, control; $\square$, pentobarbital + stimulation.

was added directly to the incubation medium during the uptake process. Administration of pentobarbital to rats had no effect on the uptake of low concentrations of 3H -glutamate or 3H -tyrosine by hippocampal synaptosomes, indicating that the reduction in choline uptake was not a non-specific effect on synaptosomal uptake in general. Since there are large post-mortem increases in choline levels¹⁶, it seemed possible that the changes in V_{max} observed here could have been caused by isotopic dilution of labelled choline by endogenous choline, which may be produced to a greater extent in pentobarbital-treated and lesioned animals than in controls. When endogenous choline levels in the media were measured after incubation, however, they were extremely low and no difference was found between synaptosomal fractions from pentobarbital-treated animals and those from control animals. It also seemed possible that our results could have been caused by an increased efflux of 3H -choline or 3H -ACh from synaptosomes from treated animals, rather than by a decreased uptake. But, the efflux of radioactivity from resuspended, prelabelled synaptosomes (prepared for routine uptake) from treated or control animals showed no differences. The reversal of the pentobarbital effect on V_{max} by stimulation was restricted to the hippocampus, and occurred in no other brain regions. This indicates that the reversal was dependent on anatomical connections for carrying the impulses and was not caused by nonspecific effects of surgery.

The effect of pentobarbital administration on the low

Table 1 Alterations in high affinity choline uptake into rat hippocampal synaptosomes

Treatment	$K_m(\mu\text{M})$	$V_{\max}$ (pmols per 4 min per mg protein)
None	0.76 ± 0.08 (5)	59.8 ± 4.0 (5)
Acute septal lesion	0.84 ± 0.04 (4) [‡]	35.8 ± 2.4 (4)*
Pentobarbital	0.95 ± 0.08 (5) [‡]	38.4 ± 2.9 (5) [†]
Pentobarbital + septal stimulation	0.75 ± 0.03 (5) [‡]	59.6 ± 3.6 (5) [‡]

See legend to Fig. 1 for experimental details. Values are mean $\pm$ s.e.m. (n = number of experiments.)

* 40% reduction compared with untreated controls, $P < 0.05$.

† 36% reduction compared with untreated controls, $P < 0.025$.

‡ No significant difference compared with untreated controls.

Acute septal lesions were placed under ether anaesthesia so that anaesthetic was not present at time of death. Sham lesioned animals did not have a reduced synaptosomal uptake of choline.

affinity uptake¹⁵ of choline was also examined. With high concentrations of ³H-choline in the medium (20–150 μM), when most uptake would be by the low affinity system¹⁵, only a slight decrease (5–10%) in uptake velocity was observed in hippocampal synaptosomes derived from pentobarbital-treated animals. This change, however, could be explained on the basis of the presence of the high affinity uptake component, which would still account for a significant fraction of the choline accumulated, even at these higher concentrations¹⁵. Thus, these impulse flow-dependent changes in choline uptake are probably restricted to the high affinity system which is highly localised to cholinergic nerve terminals⁸.

While these data demonstrate an impulse-flow regulation of the accumulation of choline by cholinergic terminals, it is difficult to distinguish if the impulse-flow regulation acts primarily on the uptake mechanism or secondarily after some other regulatory change. This is because one cannot clearly separate choline uptake from subsequent acetylation of choline, that is, there is no inhibitory drug or simple treatment to block acetylation without also inhibiting uptake. On the other hand, uptake seems to be primarily affected. We have examined choline acetyltransferase in the absence of uptake, that is, in its soluble state, and have found no change in its kinetic properties with regard to substrate or cofactor. This situation is in contrast to that with the dopamine-containing neurones where kinetic changes are found in soluble tyrosine hydroxylase after changes in impulse flow¹⁷. Also, when we examined the synaptosomal content of ³H-choline and synthesised ³H-ACh from control and treated animals, we found a similar ratio of these compounds in both cases. Thus the reduction in uptake after treatment could not be explained solely by reduced conversion of choline to ACh. Also, the reduction in uptake after treatment is only observed when synaptosomes are incubated in the presence of sodium ion; this reflects the involvement of the high affinity uptake system which is highly sodium dependent^{9,15}.

Since the molecular mechanisms underlying choline uptake are unknown, we do not know how the observed changes in $V_{\max}$ occur. Because the change is in uptake velocity rather than affinity, it may be that the net result of reducing impulse flow is to either partially immobilise or slow the presumed choline carrier. Since choline uptake is dependent on ions, most notably sodium^{9,15}, changes in ionic fluxes across the membrane during impulse flow may in some way be responsible for the regulatory influence.

The results presented here show that changes occur in the high affinity choline uptake system after treatments which alter the activity of cholinergic neurones. The reduction in $V_{\max}$ in conditions where impulse flow is presumably stopped or slowed, and its increase after stimulation, would have functional significance in that the high affinity choline uptake system would respond to the need of the neurone for

formation of releasable ACh. This possibility receives additional support from experiments with perfused ganglia where nerve stimulation accelerates choline uptake by cholinergic terminals^{18,19}. These results provide strong support for the view that choline uptake is rate-limiting^{9–12} and regulatory in the synthesis of ACh.

This research was supported by grants from the US Public Health Service and the John A. Hartford Foundation.

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Dopamine-like effects of cholera toxin in the central nervous system

It is now well established that cyclic AMP acts as an intracellular 'second messenger' in mediating the effects of several hormones on their target cells¹. The hypothesis that cyclic AMP serves a similar function in mediating the effects of certain neurotransmitters in the central nervous system (CNS) has received some support^{2,3}. For example, the neurotransmitters noradrenaline and dopamine stimulate the synthesis of cyclic AMP in tissue slices or homogenates of brain regions innervated by neurones which use these transmitters^{4–6}. Further, the actions of these transmitters on neural firing when applied by iontophoresis can be mimicked by cyclic AMP². Bilateral injections of small amounts of dopamine into the nucleus accumbens of rats stimulates locomotor activity⁸. In the present experiments small amounts of cholera toxin were injected bilaterally into the nucleus accumbens of rats and the time course of its effects on adenylate cyclase and locomotor activity were determined. The only known mechanism of action of cholera toxin (choleragen) is activation of adenyl cyclase⁷ in intact cells subsequent to its binding to a cell-surface receptor, the ganglioside GM₁ (refs 9–11). The results support the hypothesis that dopaminergic transmission in the CNS is mediated by cyclic AMP.

Cannulae were implanted bilaterally into the nucleus accumbens septi (NAS) of adult male Sprague-Dawley rats and choleragen (1 μg in 1 μl) or 1 μl of vehicle was injected. To distinguish between possible pre- or postsynaptic effects of the toxin on dopaminergic mechanisms, a further group of animals was prepared which had selective bilateral lesions of the dopamine terminals in the nucleus accumbens induced

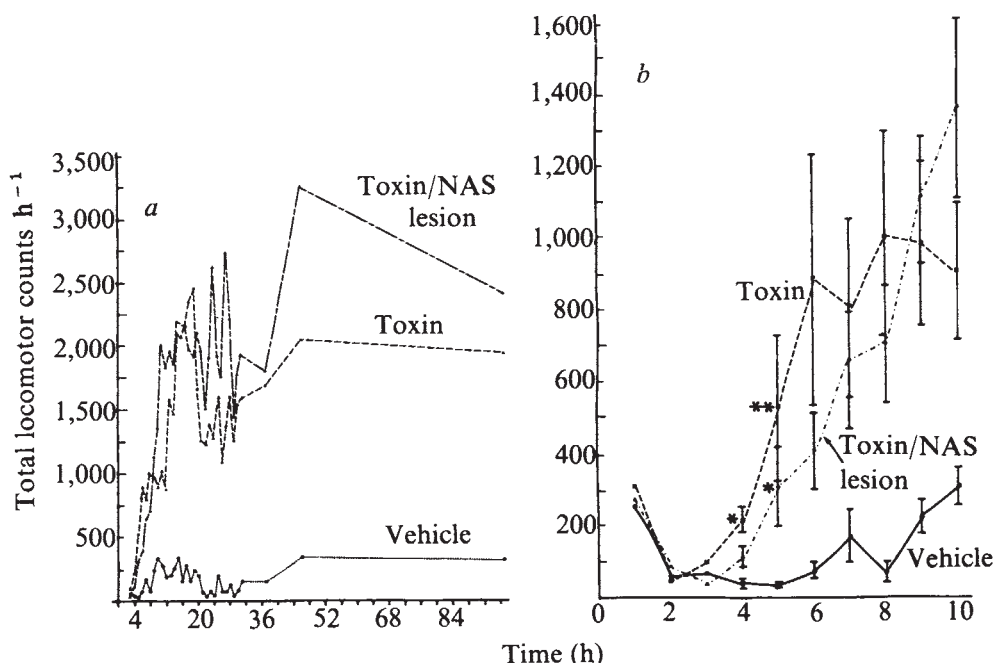


Fig. 1 *a*, Effect of cholera toxin on locomotor activity. Three groups of six adult male Sprague-Dawley albino rats were used. One group (Toxin) received bilateral injections of cholera toxin (1 μ g in 1 μ l) into the nucleus accumbens. Another group (Vehicle) was similarly treated with injections of vehicle solution (0.125 M Tris-HCl, pH 7.4, containing 0.05 M NaCl and 0.00025 M EDTA). A third group (Toxin/NAS lesion) had been previously treated with 6-hydroxydopamine (8 μ g in 2 μ l) injected one month previously bilaterally into the

nucleus accumbens to destroy the dopaminergic innervation before toxin treatment. Toxin was prepared according to the method of Finkelstein and Lo Spalluto¹⁵. Injections of cholera toxin or vehicle were made at a rate of 1 μ l min⁻¹ through 30 gauge stainless steel tubes inserted into chronically implanted guide cannulae (23 gauge stainless steel) which had previously been stereotactically implanted in the NAS. Locomotor activity was measured in a bank of wire cages each with two horizontal photocell beams along the

long axis. One animal was placed in each cage. The number of photocell beam interruptions per hour was recorded at hourly intervals for 30 h. Thereafter the animals were replaced in their home cages. Locomotor activity was recorded intermittently for a further period of 14 days. *b*, Shows an expansion of the first 10 h of (*a*). Locomotor activity in the toxin-treated groups becomes significantly activated above control activity at the fourth and fifth hours after cholera toxin injection. * $P < 0.05$, ** $P < 0.01$.

by 6-hydroxydopamine injections (8 μ g in 2 μ l) one month before cholera toxin administration. This procedure reduces nucleus accumbens dopamine concentration to approximately 20% of control, whereas striatal dopamine is not affected (P. H. K., P. Seviour, and S. D. Iversen, in the press). These animals also had cannulae bilaterally implanted and received cholera toxin (1 μ g in 1 μ l) bilaterally. After injection rats were placed in individual photocell cages and locomotor activity recorded every hour for the first 36 h and then at periodic intervals. No effect was seen for the first 3 h, after which time locomotor activity in the lesioned and non-lesioned groups of animals receiving cholera toxin rapidly increased to levels approximately ten times greater than those observed in control animals receiving vehicle injections (Fig. 1). Locomotor stimulation was still present 4 d after the injection. In fact some animals still showed significantly elevated locomotor activity 2 weeks after cholera toxin injection. As the locomotor activity seen in lesioned animals is not significantly different from normal animals receiving cholera toxin it is probable that the observed effects are postsynaptic.

It is possible to see activation of adenylyl cyclase in disrupted membranes from cells previously treated with cholera toxin¹². Adenylyl cyclase activity was therefore measured in homogenates of NAS of rats previously treated with cholera toxin (Fig. 2). After 2 h the activity of adenylyl cyclase in accumbens homogenates was not significantly different from that in vehicle-treated animals. At 5 h, however, when stimulation of locomotor activity was apparent, adenylyl cyclase activity was also elevated. Moreover, at 24, 48 and 96 h after cholera toxin, when locomotor activity was further stimulated, adenylyl cyclase activity was also further increased. Thus the effects of cholera toxin on locomotor activity and adenylyl cyclase activity exhibited similar time courses, showing in each case a characteristic lag period, similar to that observed in other cells after exposure to toxin¹²—for example, stimulation of lipolysis in isolated adipocytes by cholera toxin shows a lag of approximately 90 min¹². The locomotor effects after cholera toxin contrast with those observed after dopamine injection into the nucleus accumbens, when locomotor stimulation is immediately apparent and persists for only about 5 h⁸. As mentioned

Table 1 Adenylyl cyclase activity (pmol per mg protein in 2.5 min) in different brain areas of control and toxin-treated rats at different times

Area	5 h	Control 48 h	96 h	5 h	Toxin 48 h	96 h
Striatum	206,178	—	106,188	108,172	—	388,564
Olfactory tubercle	—	210,168	—	—	396,466	—
Hypothalamus	112,138	—	116,152	128,158	—	88,138
Cortex	128,146	—	162,156	122,162	—	118,202

Toxin (1 μ g μ l⁻¹) or vehicle was injected bilaterally into the nucleus accumbens. Each figure represents one animal (duplicate determinations). Striatum, olfactory tubercle and cortex were pooled in each animal. Dissections were carried out according to the methods of Glowinski and Iversen¹⁸ and Horn *et al.*¹³. Adenylyl cyclase was assayed as described previously¹³.

above, the effects of cholera toxin in some animals lasted for up to 2 weeks. The duration of the effect may be related to the turnover time of adenylyl cyclase molecules in the neuronal membrane. Other effects, such as an induction of phosphodiesterase postsynaptically, cannot be ruled out.

We also investigated the effects of added dopamine on adenylyl cyclase in nucleus accumbens homogenates from toxin-treated animals (Fig. 3). As previously reported¹³, dopamine (10^{-4} M) approximately doubled adenylyl cyclase activity when added to nucleus accumbens homogenates from vehicle-treated rats. Two hours after toxin treatment the effect of dopamine did not differ from that in control animals. After 5 h, however, when both locomotor activity and adenylyl cyclase activity were stimulated, dopamine was able to increase the adenylyl cyclase activity still further. The absolute increase in adenylyl cyclase activity produced by 10^{-4} M dopamine 5 h after toxin was significantly greater than the increase produced by 10^{-4} M dopamine after 2 h or in control animals. A similar phenomenon has been reported by Field¹⁴ who found that the addition of noradrenaline stimulated adenylyl cyclase both in normal erythrocytes and in cells when adenylyl cyclase activity had been increased by previous exposure to cholera toxin. The absolute increase in enzyme activity found with noradrenaline after cholera toxin treatment was greater than that found in normal cells. After 24 h, however, addition of dopamine failed to stimulate adenylyl cyclase in nucleus accumbens homogenates above toxin-stimulated levels. Various explanations are possible for this phenomenon. By this time, for example, a maximal stimulation of adenylyl cyclase activity in the heterogeneous cell population may mask the increase caused by dopamine. Alternatively dopamine may not further stimulate adenylyl cyclase if this enzyme is already maximally activated. It is also possible that long term stimulation of postsynaptic dopamine receptive neurones by cholera toxin leads to a reciprocal desensitisation of the postsynaptic dopamine receptors.

To determine how closely the activation of adenylyl cyclase in the nucleus accumbens correlated with locomotor stimulation, adenylyl cyclase was assayed in other brain regions at various times after cholera toxin injections (Table 1). Adenylyl cyclase activity in homogenates of hypothalamus and cerebral cortex was unchanged at 5 and 96 h; 48 h after

Fig. 2 Effect of cholera toxin ($1 \mu\text{g}$ in $1 \mu\text{l}$) injected bilaterally into the nucleus accumbens on adenylyl cyclase activity in homogenates of this brain region at various times after the injection. The nucleus accumbens was dissected out according to the method of Horn *et al.*¹³ and adenylyl cyclase activity measured as described previously¹³. Points are means of at least three separate incubations of at least four separate nuclei accumbens.

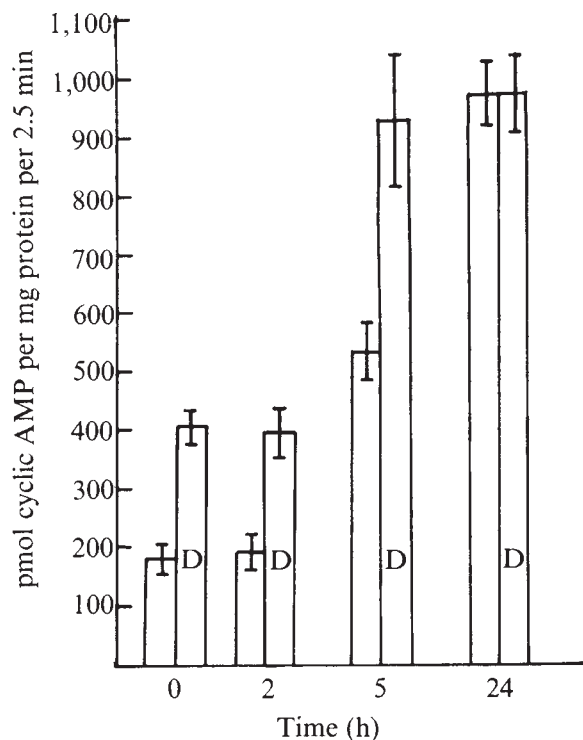
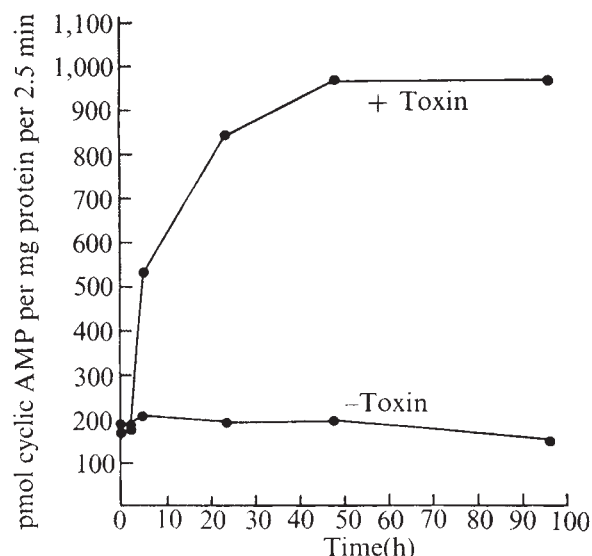


Fig. 3 Effect of dopamine (10^{-4} M) on adenylyl cyclase in homogenates of the nucleus accumbens of rats pretreated with bilateral injections of cholera toxin ($1 \mu\text{g}$ in $1 \mu\text{l}$) at various times after the injection. Adenylyl cyclase assays were carried out as in Fig. 2, except that some assay tubes contained 10^{-4} M dopamine (D). Values represent means $\pm$ s.e.m. of at least duplicate incubations of tissue from four or more animals. The increase in adenylyl cyclase observed at 5 h with dopamine is significantly greater than that observed at 0 or 2 h. ($P < 0.01$.)

injection, however, the olfactory tubercle showed increased activity and at 96 h the striatum did as well. Nevertheless, since locomotor stimulation was seen as early as 4 h after toxin injection it seems likely that activation of adenylyl cyclase in the nucleus accumbens correlates best with the behavioural response. Rectal temperature was not increased in toxin-treated rats up to 96 h.

It has been postulated that dopaminergic transmission is mediated by cyclic AMP. The present studies demonstrate that a dopamine-mediated behavioural syndrome in rats may be mimicked by activating adenylyl cyclase at the same locus, thus bypassing the dopamine receptors. The behavioural effects and the adenylyl cyclase activation have the same temporal characteristics. It also seems possible that cholera toxin will be a useful tool for studying other cyclic AMP-mediated phenomena in the CNS.

We thank Drs L. L. and S. D. Iversen and Mrs J. Reed for assistance. Cholera toxin was given by Prof. R. Finkelstein and Dr C. Miller. R. J. M. is an MRC Scholar. P. H. K. acknowledges his ICI Postdoctoral Fellowship.

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Domoic and quisqualic acids as potent amino acid excitants of frog and rat spinal neurones

THE finding that kainic acid (Fig. 1a) is a potent glutamate-like excitant of rat cerebral¹ and cat spinal² neurones has led us to test domoic acid (Fig. 1b), which is structurally related to kainic acid and which also occurs in certain marine algae and has similar anthelmintic properties^{3,4}. We have also compared the actions of these two compounds with those of α -allo-kainic acid (Fig. 1c), a stereo-isomer of kainic acid and a weaker excitant of rat cerebral neurones¹, and with quisqualic acid (Fig. 1d), an ascaricidal compound isolated from seeds of the plant genus *Quisqualis*⁵. Quisqualic acid is highly potent as a glutamate agonist at the crayfish neuromuscular junction⁶. Compounds were tested on rat spinal interneurones by microelectrophoresis⁷ and on frog spinal motoneurones by superfusion of the procaine-blocked⁸ hemisectioned spinal cord *in vitro*⁹. On both these preparations (see Table 1), the anions of domoic and quisqualic acids were found to be at least two orders of magnitude more potent than L-glutamate, and equal to or stronger than kainate, whereas α -allo-kainate was a considerably weaker excitant.

The high activity of quisqualate extends the range of structures which can replace the terminal carboxymethyl group of L-glutamate with enhancement of activity. These alternative terminal groups include those given in Table 2. All of these acidic terminals contain additional electronegative atoms or groups which may promote an increased interaction of the terminal group as a whole with a corresponding electropositive region of the receptor. The high potency of quisqualic acid (one to two orders higher than that of any of the other compounds of Table 2) indicates an exceptionally effective interaction of the isoxazolidinedione moiety with this electropositive receptor site. The equally potent actions of domoate and kainate may have a similar explanation. Although these two substances have the same carboxymethyl terminal as glutamate, the considerably weaker action of α -allo-kainate, and also of dihydrokainate², indicates that the high activity of kainate and domoate is associated with unsaturation in the side chain coupled with a *cis* relationship of the C₃ and C₄ substituents. This suggests the possibility of synergistic action of the side chain unsaturation and the carboxymethyl terminal in the interaction of these parts of the molecule with the electropositive region of the receptor.

When allowance is made for the different experimental conditions used, the high potency of domoate, quisqualate and kainate in depolarising spinal neurones seems to compare with that of certain peptides related to substance P (refs 16 and 17). It is not known, however, whether the amino acids and these peptides act at the same sites. The amino acids are all closely related structurally to L-glutamic acid, and it seems likely that they act on glutamate receptor sites. Whether these are identical with the receptor sites for synaptically-released

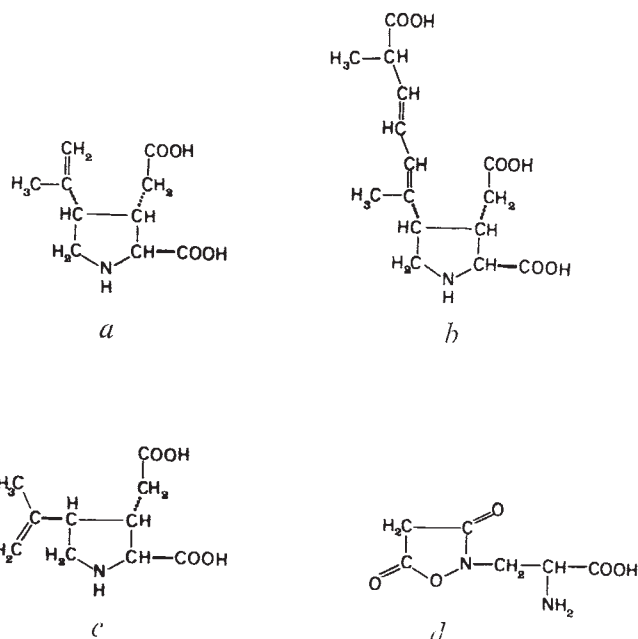


Fig. 1 Structures of kainic acid (a), domoic acid (b), α -allo-kainic acid (c) and quisqualic acid (d).

Table 1 Relative potency of amino acid excitants

Compound	Potency relative to L-glutamate = 1.0	
	Rat spinal interneurones*	Frog spinal motoneurones‡
α -allo-Kainate	1-3	0.4 (0.3-0.5; 5)
D,L-Homocysteate	3-5	11 (6-14; 10)§
Kainate	40->200†	127 (85-185; 19)
Quisqualate	25->200†	389 (151-614; 10)
Domoate	50->200†	293 (247-373; 10)

For microelectrophoretic administration, domoic, quisqualic and kainic acids were used as the monosodium salts (20 mM) dissolved in 150 mM NaCl, and α -allo-kainic acid as the monosodium salt (200 mM) in water. Monosodium L-glutamate (200 mM in water) and monosodium D,L-homocysteate (20 mM in 150 mM NaCl or 200 mM in water) were used as standard excitants. Extracellular recordings of the discharge of rat lumbar spinal interneurones were made using the 4 M NaCl-filled centre barrel of seven-barrel micropipettes. Full details of the preparation have been described⁷. Comparisons of drug potency were made by increasing the current required in each barrel to produce equal frequencies of spike discharge. The superfusion solution used for the frog spinal cord experiments had the following composition: NaCl, 111 mM; KCl, 2 mM; NaH₂PO₄, 1 mM; CaCl₂, 2 mM; Tris base, 10 mM; NaHCO₃, 10 mM; glucose, 12 mM; procaine hydrochloride, 1 mM; and 11.3 M HCl was used to adjust the solution to pH 7.9. The temperature was maintained at 13 °C. The effect of a compound added to the solution was measured from the magnitude of the potential recorded between the distal end of ventral roots 8 or 9 and the bathing solution. This potential was generated by changes in motoneurone membrane potential, electrotonically propagated along the ventral root¹⁰. Procaine was included in the perfusion medium to block conduction in the preparation, thereby preventing the indirect effects of the test substances on motoneurones which could otherwise arise through actions on interneurones. Tetrodotoxin (10⁻⁷ M) was also used for this purpose in some experiments, and gave identical results. The test substances all caused motoneuronal depolarisation. Dose-response curves were obtained by the addition of the substances to the medium in various concentrations.

*Microelectrophoretic application. Inverse ratios of ejection currents required to achieve equal firing frequencies. Multiple comparisons on eight interneurones in two animals.

†In some instances, merely reducing the retaining currents for the three strongest excitants was sufficient to achieve a firing rate equal to that obtained by 20-60 nA L-glutamate (200 mM) or of D,L-homocysteate (20 mM in 150 mM saline). Only minimum potency ratios could be calculated in these cases.

‡Calculated by comparison of ventral root depolarisation caused by various concentrations of excitants with the log dose-response curve for L-glutamate. Comparisons made on three frog cords; range and number of observations in parentheses.

§Taken from earlier studies.

Table 2 α -Acidic terminals of excitatory amino acids

General structure:	Compound	Reference
$\begin{array}{c} \text{H}_2\text{N} \\ \\ \text{HO}_2\text{C}-\text{CH}-\text{CH}_2-\text{R} \end{array}$		
R = $-\text{CH}_2-\text{CO}_2\text{H}$	Glutamic acid	9, 11
R = $-\text{CH}_2-\text{C}(=\text{O})-\text{CO}_2\text{H}$	4-Methylene glutamic acid	12
R = $-\text{CH}_2-\text{F}$	4-Fluoroglutamic acid	12
R = $-\text{CH}_2-\text{SO}_3\text{H}$	Homocysteic acid	13
R = $-\text{S}-\text{SO}_3\text{H}$	S-Sulphocysteine	*
R = $-\text{CH}_2-\text{SO}_2\text{SH}$	2-Amino-4-thiosulphonylbutyric acid	†
R = $-\text{NH}-\text{CO}-\text{CO}_2\text{H}$	β -N-Oxalyl- α , β -diaminopropionic acid	14
	6-Hydroxy-2-pyridylalanine	†
	2,4,5-Trihydroxyphenylalanine (6-Hydroxy-dopa)	†
	Ibotenic acid	15
	Quisqualic acid	This paper

*D. R. Curtis, D. Felix and J.C.W. (unpublished).

†R.H.E. and J.C.W. (unpublished).

excitatory transmitter remains uncertain. Clearly, the need for specific antagonists of synaptic and chemical excitation grows increasingly more important.

We thank Professor T. Takemoto (Tohoku University) for gifts of domoic, quisqualic and α -allo-kainic acids. Support from the MRC and the National Fund for Research into Crippling Diseases is acknowledged.

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Prolactin-stimulated production of somatomedin by rat liver

GROWTH hormone (GH) and pituitary prolactin (PP) are structurally homologous and have functional similarities both in the lower vertebrates¹ and in mammals². In the whole animal a major function of GH is to stimulate the longitudinal growth of the skeleton, and GH acts by controlling the production of a second series of hormones, the somatomedins. The somatomedins are present in serum and will stimulate the rates of division and matrix production of chondrocytes *in vitro*^{3,4}. GH itself has no effect *in vitro* on the cartilage cells, the chondrocytes.

It is now known that GH will stimulate both liver⁵ and kidney⁶ to produce the somatomedins and that in man the liver is a major site of somatomedin production⁷. If bovine growth hormone (BGH) in a chemically defined medium is perfused through rat liver, somatomedin activity is subsequently found in the perfusate; this somatomedin(s) has properties similar, in many respects, to that found in serum⁸⁻¹⁰. Pharmacological doses of BGH (1-10 $\mu\text{g ml}^{-1}$) have to be used for significant somatomedin activity to be detected in the perfusates. At the concentrations of BGH used, more physiological levels of bovine PP (10-100 ng ml^{-1}) would have been present. We have therefore measured the stimulation of somatomedin activity by PP at concentrations up to 500 ng ml^{-1} in the perfused rat liver system. Our results demonstrate that in this system PP stimulates the production of somatomedin with properties similar to that found following GH perfusion.

Livers from normal male Sprague-Dawley rats (220-260 g) were perfused as described by Dehn⁹. The liver was perfused first with 100 ml Waymouth's medium¹¹ supplemented with 0.5% (w/v) bovine serum albumin (Cohn fraction V) to wash out endogenous somatomedin activity, the final 15 ml being collected as a liver control. A further 100 ml Waymouth's medium, containing 0.5% bovine serum albumin, was then circulated three times through the same liver; this medium contained either no added hormones (control perfusion) or BGH (0-10 $\mu\text{g ml}^{-1}$) or OPP (0-500 ng ml^{-1}). Following perfusion red cells were separated by centrifugation and somatomedin activity assayed by Dehn's modification of the technique described by McConaghey⁵.

Ovine pituitary prolactin (OPP) from both sources at concentrations of 0-500 ng ml^{-1} had no significant effects on costal cartilage metabolism as measured by the rate of ³⁵SO₄ uptake, an index of cartilage proteoglycan biosynthesis (Table 1). After perfusion through rat liver, media containing either OPP (50 ng ml^{-1}) or BGH (1 $\mu\text{g ml}^{-1}$) now significantly ($P < 0.001$) stimulated ³⁵SO₄ uptake whereas media after control perfusions did not (Table 1). The optimal increase in ³⁵SO₄ uptake (Table 1) was present after perfusion with either OPP (50 ng ml^{-1}) or BGH (1.0 $\mu\text{g ml}^{-1}$). All these perfusions were carried out with livers of animals killed at 1100. In contrast perfusion with OPP (50 ng ml^{-1}) of livers of animals killed at 1500 only stimulated ³⁵SO₄ uptake marginally. Perfusion with BGH clearly stimulated ³⁵SO₄ uptake at both times (Table 1). Circadian responses to prolactin have been described for other prolactin-stimulated systems^{14,15}.

Preliminary studies on the properties of the somatomedin activity produced after perfusion of rat liver with OPP demonstrate that they are similar to those we have shown

Table 1 Stimulation of $^{35}\text{SO}_4$ uptake by costal cartilage by media containing PP, or GH, before and after perfusion through rat liver

	Uptake (c.p.m. %) compared with basal media control (hormone added at assay stage)	No. of perfusions	Uptake (c.p.m. %) compared with liver control (hormone added at perfusion stage)
Controls			
Liver control	121 ± 22 (75)	—	100 ± 15 (75)
Waymouth's medium	100 ± 7 (102)	—	82 ± 14 (102)
Ovine prolactin			
0 ng ml ⁻¹	—	3	113 ± 18 (9)
5 ng ml ⁻¹	96 ± 8 (8)	3	138 ± 28 (9)
50 ng ml ⁻¹	99 ± 10 (11)	6	187 ± 15 (15)*
50 ng ml ⁻¹ (NIH)	106 ± 7 (7)	6	266 ± 53 (17)*
50 ng ml ⁻¹ (1500)	—	6	119 ± 8 (18)†
500 ng ml ⁻¹	83 ± 8 (9)	5	171 ± 14 (15)*
Bovine growth hormone (NIH)			
100 ng ml ⁻¹	103 ± 10 (6)	2	77 ± 16 (4)
1 µg ml ⁻¹	95 ± 12 (6)	3	181 ± 24 (9)*
10 µg ml ⁻¹	121 ± 6 (5)	2	164 ± 16 (5)*
10 µg ml ⁻¹ (1500)	—	2	192 ± 27 (6)*

For each assay, costal cartilage sections (3–4 mm long) were taken from each of five Sprague–Dawley rats, each of 50–60 g; the costochondral junctions were discarded. Five cartilage sections, one from each rat, were added to 2.0 ml of the sample to be assayed and the reaction started by the addition of 1.0 µCi ml⁻¹ $^{35}\text{SO}_4$ (carrier-free from Radiochemical Centre, Amersham). Each sample was assayed in triplicate. Flasks were maintained at 38 °C for 48 h in a shaking water bath, with an atmosphere of 5% CO₂ in air. The reaction was stopped by immersion in boiling water and the uptake of $^{35}\text{SO}_4$ into cartilage proteoglycans estimated by liquid scintillation counting according to the method of McConaghey⁵. Results are expressed as c.p.m. $^{35}\text{SO}_4$ uptake per mg wet weight cartilage compared to the liver control (see text). Each figure is the mean ± s.e. for the number of assays given within the brackets. Unless otherwise stated all perfusions were carried out at 1100, using ovine prolactin (Sigma).

Significance of differences between means assessed by Student's *t* test: **P* < 0.001; †*P* = 0.05–0.02. The difference between $^{35}\text{SO}_4$ uptake after incubation in medium + 50 ng OPP/ml perfused at 1100 and the same medium perfused at 1500 is significant at *P* < 0.001.

BGH used was batch B17 from NIH (0.92 IU GH mg⁻¹ and <0.50 IU PP mg⁻¹), (ref. 12 and personal communication). OPP preparations were obtained from NIH batch S-10 (26 IU PP mg⁻¹ and <0.01 IU GH mg⁻¹) (ref. 12 and personal communication) and from Sigma, batch L-4876 (about 30 IU PP mg⁻¹). Acrylamide gel electrophoresis¹³ of these preparations demonstrated that there was less than 5% contamination of the BGH with bovine PP and of the OPP preparations with ovine GH.

after BGH perfusion^{8–10}. Thus activity cannot be replaced by the addition of serine and glutamine, which both stimulate cartilage metabolism¹⁶ and which are the only two amino acids present in suboptimal concentrations⁹ in Waymouth's medium (Table 2). In addition the OPP-induced somatomedin activity, like that induced by BGH, will stimulate $^{35}\text{SO}_4$ uptake by isolated rabbit chondrocytes in the assay of Ash and Francis¹⁰.

These results demonstrate that both preparations of OPP stimulate somatomedin production by rat liver in the *in vitro* conditions used in the present work. Could these effects of OPP be explained by contamination with ovine GH? The preparations of OPP used contain low levels of GH and are more potent than the BGH used (Table 1). Although we have so far been unable to test ovine GH directly in the liver perfusion system, it is known that ovine GH and BGH have similar potencies in both the weight-gain test in 100 g hypophysectomised rats¹² and the rat-tibial-width test¹⁷. It is thus most unlikely that the effects of OPP on liver somatomedin production are caused by contamination with ovine GH as the latter would have to be many times more potent than BGH. The distinct actions of BGH and OPP when perfused through the rat liver at different times of the day also provide evidence that each can act separately on liver to stimulate somatomedin production.

Recent clinical evidence has suggested that PP can

stimulate *in vivo* both somatomedin production (detectable in serum) and continued skeletal growth in the absence of immunologically detectable GH (refs 18 and 19). These results, together with those presented here, demonstrate that pituitary prolactin can act to stimulate somatomedin production and thus function as a skeletal growth hormone. Further work will be required to elucidate the relative importance of prolactin and growth hormone in the control of mammalian skeletal growth in normal physiological conditions.

This work was supported by a grant from the MRC to Professor R. B. Duthie. BGH was a gift from Dr A. E. Wilhelmi (NIH) and OPP from Dr D. Horrobin. We thank Dr L. E. Reichert, Jr for information on the potencies of the NIH hormones.

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Received December 23, 1974; accepted April 1, 1975.

Table 2 Properties of somatomedin activity produced following perfusion of rat liver with OPP (NIH)

	No. of perfusions	Uptake (c.p.m. %) compared with basal medium
Waymouth's medium	—	100 ± 18 (14)
OPP perfusion medium	3	166 ± 21 (9)*
Waymouth's medium + serine + glutamine	—	136 ± 13 (8)
OPP perfusion medium + serine + glutamine	3	211 ± 24 (7)*

Assays were carried out as in Table 1. Serine and glutamine were added where indicated to bring the final concentrations in the assay medium to 1.75 mM l⁻¹ and 3.5 mM l⁻¹, respectively⁹. Perfusion was carried out with OPP (NIH; 50 ng ml⁻¹).

**P* against controls < 0.02.

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matters arising

Feeding analogues

THE recent letter¹ on the control of food intake recalls the old caveat that it is very easy to make an analogue; the problem is to know what it is an analogue of.

The analogue presented invokes operation of an on-off feeding switch by the level of 'energy flow'. It is not at all clear what flow of energy is meant. The values used for turn-on and turn-off of feeding roughly correspond with those estimated by Le Magnen and Devos² as 'food utilisation', meaning rate of absorption from the gut. The block diagram, however, shows the effective energy flow after the summing point with lipogenesis-lipolysis; that is, for practical purposes, the energy flow triggering the feeding switch of the analogue is total metabolic rate. Now, for rats, the mean increment in metabolic rate from the beginning to the end of a meal (or, with sign change, from the end of one meal to the beginning of the next) is not more than 10%, and is often much less or even negative^{2,3}. It does not approach the 230% (18–60 calorie min⁻¹) proposed by Toates and Booth¹. In the case where the beginning of a meal coincides with the change from rest to activity, and general motor activity continues beyond the end of the meal, then increase in metabolic rate from just before the onset of the meal to its termination can be about 75%, but that change is almost entirely a function of the change from rest to activity with only a small contribution from feeding as such³.

We therefore seem to have the situation in which energy flow is interpreted as rate of energy absorption from the gut but is inserted into the analogue as if it were metabolic rate; or in which it is interpreted as metabolic rate but gut absorption values are mistakenly used. This is not just a quantitative discrepancy. Even if 'energy flow' denotes metabolic rate and even if realistic values for metabolic rate are used the analogue as presented would have a nonsense output. Since the major changes in metabolic rate are attributable to change between rest and activity and since feeding must be a part of activity³, feeding by this analogue can only occur during rest, when, by definition, it cannot occur.

The approximate agreement shown between real rats and the analogue for some variables could indicate that these variables are too insensitive for assessment

of the function of the model.

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¹ Toates, F. M., and Booth, D. A., *Nature*, 251, 710–711 (1974).

² Le Magnen, J., and Devos, M., *Physiol. Behav.*, 5, 805–814 (1970).

³ Morrison, S. D., *J. Physiol. Lond.*, 197, 305–323 (1968).

TOATES AND BOOTH REPLY—We stated in our second paragraph¹ that the energy flow controlling feeding in our model is that sampled by hypothetical receptors in neural tissue. As the text and figure specify, we make a crude estimate of this by summing the large and considerably variable flow of energy from the gut, with the circadian variations in net flow from or to triglyceride. We are concerned with some of the energy flows between tissues, not the energy flows between the organism and the environment. The dilemma Morrison² poses is therefore fallacious. In a later version of the model³ we allow for circadian variations in heat production by non-receptor tissues; metabolic rate data assist us there, although (as Morrison says²) such variations are relatively small. The physiological variables we use are sufficiently sensitive to give unrealistic behavioural predictions when set at values outside the observed range.

We heartily concur with Morrison's point² that the empirical interpretation of mathematical models is a treacherous business. We have been able to discuss the relationships between this model and the facts in more detail elsewhere³. We welcome this opportunity to counteract misunderstanding that a necessarily brief report almost inevitably permits.

¹ Toates, F. M., and Booth, D. A., *Nature*, 251, 710–711 (1974).

² Morrison, S. D., *Nature*, 255, 169 (1975).

³ Booth, D. A., Toates, F. M., and Platt, S. V., in *Hunger: Basic Mechanisms and Clinical Implications* (edit. by Novin, D., Wyrwicka, W., and Bray, G. A.), (Raven, New York, in the press).

Lead in children's teeth

DAY, HART AND ROBINSON¹ examined the geographical dependence of lead concentrations in urban street dust in several ways. When subdividing data according to city quadrant they noted that the four values ranged from 91 to 122% of the mean value of 970 p.p.m.

We divided our data geographically

Table 1 Lead concentrations in tooth crowns according to city zones

Zone	Mean concentrations (p.p.m.)			
	Pb	Zn	Cd	Cu
NE	71 ± 19*	132 ± 32*	4.3	6.6
SW	53 ± 16*	143 ± 36*	4.4	6.6

* Standard deviations (28 samples). Difference (north-east/south-west) significant for Pb ($t = 3.7$, $P < 0.001$).

when studying lead concentrations in crowns of permanent premolar teeth collected from Bristol school dental clinics. The data, plotted according to the home location of the children, fell equally on both sides of a line passing through the centre of the urban area and the Avonmouth industrial complex, approximately 10 km to the north-west. This orientation, however, is approximately at right angles to lead and zinc concentration contours² based on extensive sampling of soil and of elm and hawthorn leaves in connection with the regional environmental survey (Sabrina Project).

Concentrations of lead in Bristol children's teeth are typically 60 p.p.m. A mean value for tooth samples from 28 children living in a zone to the south-west of the chosen demarcation line was only 75% of that for the same number representing the zone to the north-east (Table 1). There was a non-significant difference in the opposite direction for zinc analyses; the values for cadmium and copper were the same in the two zones.

Another aspect of this type of environmental observation—but in which no geographical distinction was evident—is provided by a study³ of concentrations of lead and other metals in developing teeth in cases of stillbirth and neonatal death. There was significantly more lead in the dentitions of those born in 1972–73 than in those dating from 1957–63.

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² Little, P., and Martin, M. H., *Environ. Pollut.*, 3, 241–254 (1972).

³ Stack, M. V., Burkitt, A. J., and Nickless, G., *Postgrad. med. J.* (in the press).

Factors influencing effect of hydrocortisone on rat brain tryptophan metabolism

THE concentrations of rat brain 5-hydroxytryptamine (5-HT) and its metabolite 5-hydroxyindole acetic acid (5-HIAA) have been found to be decreased by injection of hydrocortisone¹⁻³ or other corticosteroids⁴. Benkert and Matussek⁵ did not, however, observe changes in 5-HT concentration at various times

this protocol we now find a fall of both total and free plasma tryptophan and a fall of tryptophan concentration in both liver and brain (Table 1). Brain 5-HT decreased but not significantly, while 5-HIAA was considerably and significantly lowered (Table 1).

● Brain tryptophan, 5-HT and 5-HIAA are all unaltered following hydrocortisone (5 mg kg⁻¹) administration to gerbils⁷. In these animals (unlike the rat) the hepatic enzyme tryptophan pyrrolase is not induced by hydrocortisone^{7,8}. This is consistent with an association between

liver homogenates after hydrocortisone treatment, and has now been demonstrated in the perfused liver. Thus, kynurenine production from tryptophan by isolated perfused rat liver preparations is much lower in older animals both before and after hydrocortisone (5 mg kg⁻¹) pretreatment 3 h previously (Table 2).

Thus we have shown that hydrocortisone can alter tryptophan metabolism both peripherally and in the brain and confirm that changes are consistent with decreased brain 5-HT synthesis. These changes are,

Table 1 Effect of hydrocortisone on tryptophan metabolism in rats

Injected	Plasma tryptophan (µg ml ⁻¹)		Tissue tryptophan (µg g ⁻¹)		Brain 5-hydroxyindoles (µg g ⁻¹ wet weight)	
	Total	Free	Liver	Brain	5-HT	5-HIAA
Saline	27.04 ± 2.05	3.92 ± 0.84	4.89 ± 0.51	2.04 ± 0.19	0.45 ± 0.05	0.87 ± 0.12
Hydrocortisone	22.77 ± 3.38*	1.84 ± 0.51†	3.66 ± 0.35†	1.45 ± 0.09‡	0.38 ± 0.05	0.51 ± 0.06‡

Male Sprague-Dawley rats (Anglia Laboratory Animals, Alconbury) 35 d old were used. Animals were killed 6 h after injection. Plasma tryptophan was measured as described previously¹⁰, tissue tryptophan by the method of Denckla and Dewey¹¹ and brain 5-HT and 5-HIAA by the method of Curzon and Green¹². Results expressed as mean ± s.d. of six observations. Different from saline injected controls.

* $P < 0.05$.

† $P < 0.01$.

‡ $P < 0.001$.

Table 2 Influence of age on effect of hydrocortisone on kynurenine production by isolated perfused rat liver

Age (d)	Injected	Hepatic kynurenine production (µg ⁻¹ g ⁻¹ h ⁻¹)
35	Saline	35 ± 5(4)
	Hydrocortisone	139 ± 32(4)*
100	Saline	22 ± 8(4)†
	Hydrocortisone	65 ± 31(5)*‡

Livers were perfused by the method of Hems *et al.*¹³ with a semi-synthetic medium¹⁴ containing tryptophan (1.0 mM). Rats were injected with hydrocortisone (5 mg kg⁻¹) or saline 3 h before the start of the liver perfusion. Results expressed as mean ± 1 s.d. with number of observations in brackets.

* Different from saline-injected control rats of same age $P < 0.01$.

† Different from saline-injected 35-d rats $P < 0.05$.

‡ Different from hydrocortisone-injected 35 d rats $P < 0.01$.

after injecting hydrocortisone acetate. More recently, Hillier *et al.*⁶ reported no change of 5-HT but a rise of 5-HIAA 3 h after hydrocortisone (15 mg kg⁻¹ intraperitoneally). The latter authors also found no change in serum total tryptophan and an increase in free serum tryptophan at this time. The following observations may clarify these contradictory findings.

● Using the hydrocortisone dose of Hillier *et al.*⁶ of 15 mg kg⁻¹, we confirm that there is no change in total plasma tryptophan 3 h after injection of the steroid, but also found no alteration in free tryptophan. In the original work however^{1,2} 5 mg kg⁻¹ hydrocortisone (as the sodium succinate derivative) was injected intraperitoneally and brain 5-HT and 5-HIAA determinations were made 6 h later, at which time the decrease in both compounds was maximal. Using

the increased hepatic pyrrolase activity in rats following hydrocortisone² and the changes in tryptophan metabolism now reported.

● Other factors may also be important when studying the effects of hydrocortisone on tryptophan metabolism. The time courses of hydrocortisone actions depend on the form in which it is injected. Hydrocortisone sodium succinate leads to more rapid changes of pyrrolase activity than the less water soluble hydrocortisone acetate or free steroid⁹.

● The age of the animals is also important. Older animals (100 days or more) do not exhibit the brain 5-hydroxyindole changes or the large hepatic pyrrolase induction shown by younger rats (35 days; 120 g) following the same hydrocortisone dose per kg body weight⁹. This smaller increase of pyrrolase activity in older animals was previously demonstrated by measuring enzyme activity in

however, dependent on several factors; times of observation, age of animals and the hydrocortisone preparation used. Though it would be rash to extrapolate too readily from acute pharmacological experiments to a chronic illness such as endogenous depression, the possibility exists that similar changes have significant roles in the aetiology of psychiatric disorders in some subjects^{1,2} or, indeed, in the 5-HT metabolism of normal subjects in certain circumstances.

This work was supported by the Medical Research Council. We thank Miss J. P. Hughes and Mr. P. Hutson for technical assistance.

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Matters arising

Matters Arising is meant as a vehicle for comment and discussion about papers that appear in *Nature*. The originator of a Matters Arising contribution should initially send his manuscript to the author of the original paper and both parties should, wherever possible, agree on what is to be submitted. Neither contribution nor reply (if one is necessary) should be longer than 300 words and the briefest of replies, to the effect that a point is taken, should be considered.

¹ Curzon, G., and Green, A. R., *Life Sci.*, **7**, 657-663 (1968).

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⁵ Benkert, O., and Matussek, N., *Nature*, **228**, 73-74 (1970).

⁶ Hillier, J., Hillier, J. G., and Redfern, P. H., *Nature*, **253**, 566-567 (1975).

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⁹ Green, A. R., and Curzon, G., *Biochem. Pharmacol.*, **24**, 713-716 (1975).

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Narrow-angle cosmic-ray anisotropies

SPELLER, *et al.*¹ have presented a measurement of the diurnal cosmic-ray anisotropy, as found with underground muons in London. An additional, but quite different, interpretation of their data is, however, possible.

From the widely accepted diffusion models for cosmic-ray propagation in the Galaxy, one could expect only a diurnal anisotropy (24-h wave). A newer model², based on adiabatic propagation of cosmic rays, predicts that an excess could occur within some small region of the celestial sphere. The direction of the excess would depend on the orientation (in space and time) of the source object relative to galactic magnetic field lines which connect the source with the Solar System.

A narrow-angle anisotropy near the celestial equator was reported³ and confirmed⁴ during the time of the London experiment (see Table 1). It resembled an earlier anisotropy⁵ in that it was observed at the time of a sunspot minimum, then gradually faded away. The London data are shown in Fig. 1, split into periods before, during and after the

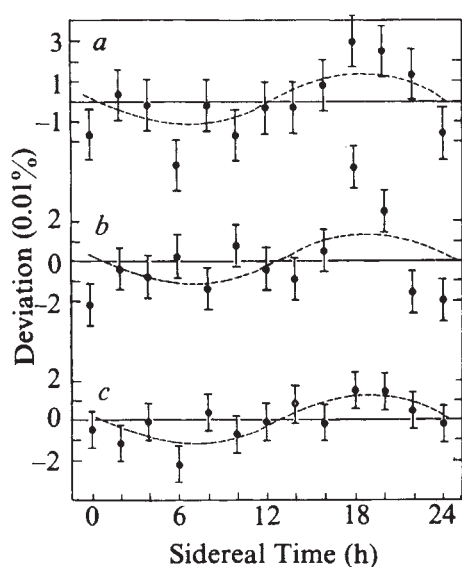


Fig. 1 The London data for: *a*, 1961-62; *b*, July 1963-June 1964; *c*, 1965-68, showing the deviation from the average intensity against sidereal time. The sine curve fit to the total data is shown. The anisotropy is present in *b*, but lacking in *a* and *c*, in agreement with ref. 3.

1964 anisotropy. The point at 18 h is $3.2\sigma_N$ (standard deviations) above the curve.

The apparent position of this peak agrees with the Winnipeg data³ if one includes geomagnetic deflections calcu-

Experiment location	Nagoya	Winnipeg	Hong Kong	London
Latitude ($^{\circ}$ N)	35	50	22	51
Period observed	1952-56	Dec. 1963-Sept. 1964	Jan. 1964-Mar. 1964	1963-64
Not observed	1957-61	Nov. 1964-Dec. 1964	—	1965-68
Declination ($^{\circ}$ N)	0.5	0 ± 18	—	—
Right ascension (h)	5.25	20.5^*	23^*	19^*
Angular aperture ($^{\circ}$)	5×7	± 18	± 45	± 63
Particle rigidity (GV)	280 ± 40	> 40	> 30	150 ± 50
Significance (standard deviations exceeded)	6	16	5	5

*Uncorrected for geomagnetic deflection.

lated for particles of 100-200 GeV. (This explanation is inadequate for the reported Hong Kong position⁴. Other factors, such as accessibility to lower declinations, may have played a role.) This estimated energy is above the thresholds of the Winnipeg and Hong Kong detectors but is near the London threshold at a zenith angle of 51° (the efficiency is about 10% at 160 GeV and 90% at 1600 GeV). The small size of the anisotropy in the London data (0.03%, by comparison with 0.3% at Hong Kong and 10% at Winnipeg) can be understood in terms of the energy threshold, the large zenith angle and the wide angular aperture.

The uncertainties σ_N shown in Fig. 1 arise from counting statistics only. The London authors argue for the use of $1.4\sigma_N$, but the scatter of their data around their sine curve in Figs 1a and c is $\sigma_s/\sigma_N = 0.89$ and 0.84 , whereas the expected scatter is $\sigma_s/\sigma_N = 0.93 \pm 0.20$. If one removes the two points at 18 h and 20 h, the other points fit well to a straight line. The two points are $5.0\sigma_N$ and $2.9\sigma_N$ above this line.

During previous sunspot minima, conditions have apparently become favourable enough to observe two narrow-angle anisotropies of rather low energy. Each would be caused by a cosmic-ray source near our magnetic field line in the Galaxy. The next sunspot minimum is expected early in 1976 (ref. 6).

I thank H. E. Bergeson for discussions and the London authors for correspondence and data. This research has been supported by NASA.

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ELLIOT AND THAMBYAHPIILLAI REPLY—We are sceptical about Barrowes's interpretation of our (London) data because: (1) We disagree with the use of $\sigma_N = 100/\sqrt{N}$ % in assessing the statistical reality of the deviation from the mean at 18 h. Our own analysis of the scatter of harmonic coefficients shows that a value of $\sigma_N = 1.8 \times (100/\sqrt{N})$ % is more appropriate than the lower limit used by Barrowes.

(2) The energy thresholds given in his Table 1 do not in themselves provide a satisfactory basis for comparing the magnitude of the effect to be expected at the various stations. To do this, it is necessary to use the complete response functions for the detectors concerned.

(3) We cannot see how a narrow-angle anisotropy can be observed at the Earth for primary cosmic rays with $E \leq 200$ GeV because of the wide spread in the deviation of such particles in the interplanetary magnetic field. The angles through which these particles would have been deflected by the field at any time during the recent solar cycle is much greater than that in the geomagnetic field.

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Messenger RNA abundance and gene regulation in eukaryotes

BISHOP *et al.*¹ found three abundance classes for poly(A)-containing mRNA in HeLa cells (17 sequences of about 8,000 copies per cell, 350 of about 440 copies and 35,000 sequences of about 8 copies). This situation may be quite general. Green, Goldberg and Todaro² found three ranges of relative collagen synthesis rates (1.5-14%; 0.15-0.4% and beyond 0.002%) and even where only one or two abundance classes are found, non-poly(A)-containing mRNA may contain another abundance class³.

A rather simple control system for gene regulation, similar to one previously proposed^{4,5}, may explain this rather reduced number of mRNA

¹ Speller, R., Thambyahpillai, T., and Elliot, H., *Nature*, **235**, 25 (1972).
² Barrowes, S. C., *Astrophys. J.*, **177**, 45 (1972).
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abundance classes which should correspond to three possible degrees of gene activation. Histones usually block DNA transcription, but specific activators (probably non-histone nuclear proteins) controlled by some interrelated gene triggers, may open different DNA portions to transcription by combining with "promoter" regions. This would be the first control level. Within the unblocked DNA portions a second control level could act, based on less stringent repressors (of the type found in bacteria) also controlled by triggers of interrelated genes. High abundance mRNA should correspond to unblocked-derepressed structural genes, medium abundance mRNA to unblocked-repressed structural genes. The many very low abundance mRNA sequences may correspond to accidental transcription of blocked structural genes during DNA replication, which may also be required for unblocking DNA portions in cell differentiation ("quantal mitosis"⁶). Alternatively, "switching on" of histone acetylation and phosphorylation may produce a general weakening of the histone block on transcription, allowing the appearance of the very low abundance mRNA class.

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² Green, H., Goldberg, B., and Todaro, G. J., *Nature*, **212**, 631 (1966).

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Structure of graphite

ERGUN¹ has suggested a structure for graphite based on new X-ray diffraction data, in which two of the carbon-carbon bond lengths in the six-membered ring of any layer are shorter than the other four. This gives rise to the so-called 1,4 quinoid structure. The atomic positions in this structure are

$$(0,0,0), (x, \bar{x}, 0), \left(\frac{1}{2} + \frac{x}{2}, \frac{1}{2} - \frac{x}{2}, \frac{1}{2}\right), \left(\frac{1}{2} - \frac{x}{2}, \frac{1}{2} + \frac{x}{2}, \frac{1}{2}\right)$$

with $x = 0.318$. The resulting square of the geometric structure factor is then¹:

$$F^2 = 16 \cos^2 \pi x (h-k) \cos^2 (\pi/2) \times [(1-x)(h-k) + 1]$$

(If the value of x is $\frac{1}{3}$, then the commonly accepted graphite structure results. That

structure comprises regular hexagonal nets, stacked in the sequence ... [AB] ...) Ergun used his measured values of the four observed ($h h 0$) reflections (the F^2 values of which, according to the equation shown here, are independent of the parameter x) to obtain a function that he did not publish but which he used to reduce the other seven observed ($h k 0$) intensities to an F^2 basis. The value for x of 0.318, which was apparently obtained by trial and error, was then used to calculate values for the remaining seven F^2_{hk0} values. The agreement between F^2_{obs} and F^2_{calc} is astonishingly good, with

$$R_2 = 100x \Sigma |F^2_{obs} - F^2_{calc}| / \Sigma F^2_{obs} = 1.25\%$$

Ergun stated that "it was evident [on the basis of his observation of sharp diffraction maxima] that the lattice is hexagonal", by which he meant $a = b$ with an angle of 120° between them. The symmetry of his structure is, however, not hexagonal, but orthorhombic. Thus, not all reflections having identical spacings have the same calculated values of F^2 . In particular, reflections having the same value of $(h^2 + hk + k^2)$ will occur at identical scattering angles, but not all of these are symmetrically equivalent.

For example, the reflection (210), with $F^2_{calc} = 1.075$, $F^2_{obs} = 1.08$ (Ergun's figures) occurs at the same diffraction angle as $(3\bar{2}0)$ and $(3\bar{1}0)$, and the calculated F^2 values of these two reflections, as given by the equation already shown, are 0.449 and 1.184. The average relative value of F^2_{calc} is thus 0.903. The adjacent reflection, (200), with $F^2_{obs} = 0.80$, is, in Ergun's table, in excellent agreement with $F^2_{calc} = 0.804$. This reflection is actually, however, the weighted average of (200) and $(2\bar{2}0)$, and the latter, with $F^2_{calc} = 1.184$ leads to a relative F^2_{calc} of 0.931. The calculated F^2 ratio for (200)/(210) is thus 1.031 instead of the 0.748 given by Ergun, and is no longer in almost exact agreement with the observed ratio of 0.741.

Because Ergun did not report his raw intensity data, but only those "corrected" with an unknown function it is not possible to use them to come to any further conclusions regarding the structure of graphite. But in any case it is clear that no support for the quinoid structure is provided by the spectacular agreement between the values of F^2_{calc} and the F^2_{obs} , which now becomes merely interesting.

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¹ Ergun, S., *Nature phys. Sci.*, **241**, 65 (1973).

Environmental modification of a genetically based behaviour pattern in *D. melanogaster*

THRELKELD *et al.*¹ have shown that the reduced mating success of mutant yellow males with normal females of *Drosophila melanogaster*² can be alleviated by genetic selection. It is important to realise that genetically based differences in behaviour are not rigid but are contingent on the environment in which they are measured.

In the present study to determine the environmental modifications necessary to increase the mating success of yellow males, two inbred strains of *D. melanogaster* were used, one wild type and the other yellow. All matings were conducted at 25°C under continuous light and were always between virgin 1-2-d-old wild-type females and yellow males. Each pair of flies was anaesthetised with ether, placed in a vial (23×85 mm) containing 10 ml of yeasted propionic acid medium and left for 9 d. A mating was scored as successful if progeny were present at the end of this time.

Table 1 Percentage mating success

Sex ratio	Sample size	Mating success
1:1	249	0.8
1:2	64	1.6
1:3	37	21.6
1:4	78	21.8
1:5	53	9.4

The sex ratio was varied from 1:1 to 1:5 (female:male). The results (Table 1) reveal significant differences between the 1:1 ratio and both the 1:3 ($P < 0.001$) and the 1:4 ($P < 0.001$) ratios. These remain significant even if mating successes are compared on a per male basis. It would seem that mating success is not a simple linear function of the number of males present and that a highly favourable interaction effect occurs when the ratio of males to females is three or four to one.

Artificial selection for seven generations was able to increase the competitive mating success of yellow males from 36% to 67% (ref 1). A change in sex ratio can increase non-competitive mating success from less than 1% to 22%. Environmental modification can produce significant changes in genetically based behaviour patterns.

This work was supported by a Scholarship from the National Research Council of Canada.

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² Bastock, M., *Evolution*, **10**, 421-439 (1956).

reviews

THE biography* of a leading theologian would not ordinarily find reviewing space in the columns of *Nature*. But there was nothing ordinary about Charles Raven either as a theologian or as a man. What other Regius Professor of Divinity at Cambridge (or anywhere else) has, while in office exercising his normal academic duties, produced a monumental biography of a man of science? The man of science was, of course, John Ray, the first and perhaps the greatest, British naturalist, who produced the first accounts of county flora in 1660 and a comprehensive *History of Plants*. If for nothing else, we owe Charles Raven's own biographer, Dr F. W. Dillistone, an immense debt for reminding us of the devoted and meticulous research with which Raven brought his task to its accomplishment when he published *John Ray: Naturalist* in 1942. Raven, so Dillistone tells us, collated every record of Ray's travels and research, translated countless descriptions and identifications from Latin and Greek, and collected nearly all the plants, birds and insects that Ray records—often from the same localities.

The resulting classic biography was itself the work of a unique man and Dr Dillistone's immensely readable, sensitive and thoughtful account of Dr Raven's life and work takes the veil off Raven's mind, taking us as far as we are ever likely to be able to penetrate into that vast treasure house. Dillistone shows us how Raven's bond of sympathy with Ray lay in their both sharing a vision of the unity of scientific and religious experience, that vision partly expressed in the title of Ray's widely influential *The Wisdom of God in the Works of Creation* (1691).

Raven was a born naturalist—observant, sharp-eyed, patient (able to wait

for long hours in atrocious weather for his observations)—a beautiful illustrator and draughtsman (learnt from his mother), an accomplished Latinist (undaunted, indeed revelling in, the Linnean scheme) and an avid collector, with an accurate and comprehensive memory. Dillistone shows how the zest for living creatures never left Raven and how his joy and thrill in the careful observation of nature was fundamental to his total vision. He takes us through Raven's formative years at home and

the early years of this century. This, so Dillistone judges, and those of us who remember Raven recalling his experience of Bateson's lectures will agree, was one of the chief goads to Raven's intellectual quest through his life—the interpretation of evolution as a non-deterministic and creative process which is the clue to the natural emergence of the human person. The consummation of this process Raven saw in a transmutation of human personality by the illumination and extension

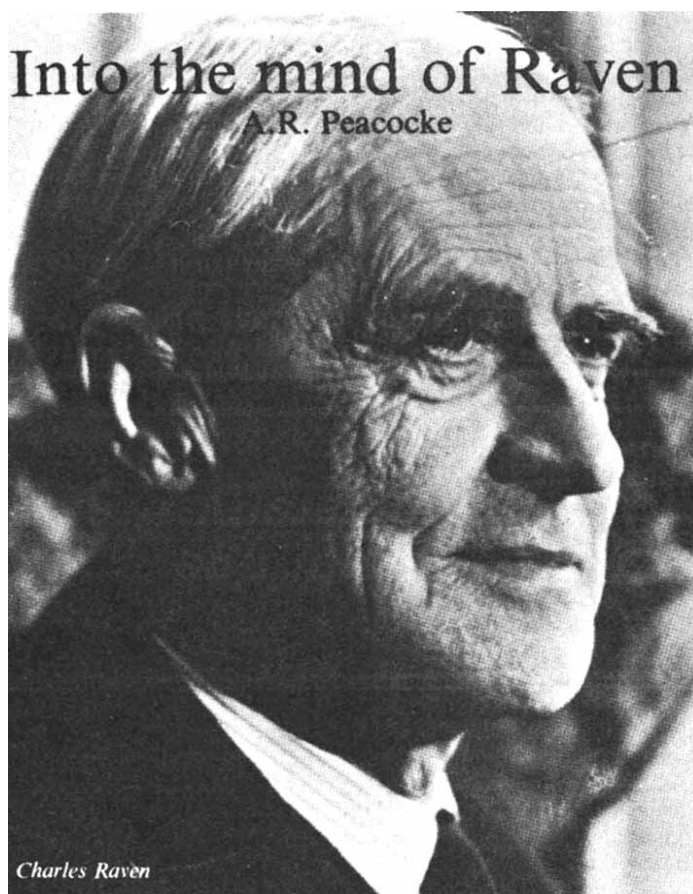
of human existence, which the Christian faith provided through its focus on the man in history in whom it was first achieved and who thereby opened the way for others.

By enriching this biography with a careful assessment of the context of thought within which Raven moved at various stages of his life, Dr Dillistone has provided us not only with an account of an exceptionally gifted man, but also with a chronicle and diagnosis of the ebb and flow of that Christian liberalism of which Raven, along with William Temple, was one of the most brilliant exponents. For this reason, this biography comes at an especially opportune time, when the near-collapse of radical politicised versions of Christianity, at least among students in this country, has left the enquiring with apparently only one other alternative—that of a conservative biblicism which

Raven regarded as an

affront to the intellectual integrity of any critically enquiring youthful mind ready to apply the same standards of judgment to its religion as to other intellectual enquiries, including that of science.

With Raven lecturing, writing and preaching in Cambridge, a critical, enquiring, exploring, yet at the same time, integrating and visionary Christian outlook was clearly available as an alternative to such conservatism, and Dillistone reminds us how science and mathematics students responded to it; men whose subsequent careers were



at the University of Cambridge and makes it clear to those of us who knew Raven only at a distance how it was that a student of first the classics and then divinity could have had such a zest for and sympathy with the natural sciences. Apparently this zest and sympathy originated from his mother's training, so that perhaps we are less surprised when Dillistone tells that while working for the Divinity Tripos, Raven attended the Lectures of William Bateson, who was one of the outstanding exponents, with a deterministic emphasis, of the new genetics during

**Charles Raven: Naturalist, Historian, Theologian*. By F. W. Dillistone. Pp. 438+18 photographs. (Hodder and Stoughton: London, 1975.) £5.25.

as different as those of Charles Coulson (the theoretical chemist who became Rouse Ball Professor of Applied Mathematics at Oxford) and Ian Ramsey (who became Nolloth Professor of the Philosophy of Religion at Oxford and later Bishop of Durham). Dr Dillistone's biography is opportune because the conservative, intellectually uncritical kind of Christianity from which Raven released so many, is today attractive in its simplistic unambiguity to many of the young who can scarcely hear any other voice. "Raven, thou shouldst be living at this hour", but, failing that, we can again catch those rich and profound cadences through his faithful biographer.

But Dr Dillistone is no adulatory sycophant. He shows us where this idol had feet of clay: his failure to understand the crises of continental thought under the impact of the Nazis; his over-magisterial non-suffering impatience with 'fools'; his too-rapid dismissal of any dialectical, non-unified system of thought; his almost pathological concern with his supposed rejection by "the Establishment" of his day on account (he supposed) of his unpopular pacifist views and of his support for the ordination of women.

More positively, Dr Dillistone has dug deeply to discover accounts of Raven's moments of illumination which carried him through the devastating experiences at the Front in the first World War and through tragic bereavements at critical moments in his life. The inner life of the Regius Professor, the Master of a Cambridge college and the Vice-Chancellor of that university, are gently, surely and shrewdly unveiled for us in a way which shows how what Raven loved to call "the sublime law of sacrifice" (quoting Fabre) lay behind the public image.

Those of us who have known Raven through his writings can, with the help of Dr Dillistone, now meet him as the complex, exceptionally gifted and contradictory man he was. Even more important, those who have known neither him or his writings can, through this sympathetic biography, now be inducted into the thought of a man who combined enthusiasm for science with insight into religious experience into a unique amalgam so clearly needed by our disrupted and fissiparous culture. For Raven, in both spheres, the exploration was liberal and open-ended and productive of that unity of vision shared, across the centuries, with Ray.

of tabulation of characteristics and by a discussion of the validity of the status of the species groups in comparison with the conclusions of other workers. In the succeeding two chapters this process is repeated for the actinomycete and bacterial floras of other habitats, notably the gut and faecal communities of the larvae of *Bibio marci*, an important component of the fauna active in soil decomposition, and the rhizoplane community of *Robinia pseudo-acacia*. These three chapters comprise two thirds of the total text; they are almost wholly devoted to the reporting and discussion of the taxonomic studies. This gives a highly specialised character to the book that seems out of keeping with the drift of the early chapters and the stated aim.

The interest of the same readers may, however, be reclaimed by the contents of the last chapter. Here, there is an enormously stimulating discussion ranging over many aspects of the structure and functioning of the microbial communities of soil, including such topics as microbial successions, the role of microbes in soil humification and fertility, the dissemination of soil microbes, and the maintenance of the genetic diversity of soil populations. Here also the ecological implications of the work described in the three main chapters is allowed to struggle free of its taxonomic clothing and to be seen in a wider ecological context.

I was left with a strange impression that I had read two books in one. Anyone with an interest in the microbial ecology of the soil who can gain access to the book will find much to stimulate him in the early chapters and in Chapter Seven. Whether they will find it worth paying the expensive price for the sake of the specialised remainder will depend on their own particular interests. A number of reservations must also be recorded. I found it difficult to be convinced by much of the experimental work in Chapter Three; for instance the relevance of survival testing of microbes over the temperature range 35–65 °C is obscure when related to their activity in a forest soil of temperate climate—particularly when no suggestion is made of potential thermophilic phases. The lack of detail in the reports of the quantitative ecological data contrasts strongly with the admirably complete treatment of the taxonomic data and detracts from the impact of the topics discussed in Chapter Seven.

Nevertheless, this well-produced book remains an important contribution to the literature, partly to the specialised field it serves but more generally because of the opportunity it affords for an appraisal of the Szabo philosophy of "intensive, single-site study".

Mike Swift

Not whole but just some of the parts

Microbial Communities in a Forest-Rendzina Ecosystem: The Pattern of Microbial Communities. By I. M. Szabo. Pp. 415. (Akademiai Kiado; Budapest, 1974.) £10.50.

THE microbial ecology of soil is a subject which has in the last few years acquired a voluminous literature at an ever increasing rate. Unfortunately, this has been achieved without any comparable increase in the understanding of the systems under study. The diversity of microbial communities, their functions and their microhabitats have obscured all attempts to derive any but the most basic ecological generalisations. Professor Szabo attributes this failure, in part, to a dispersal and dilution of research effort. In spite of the complex nature of the system (or perhaps because of it) research planning has tended to concentrate on specific functions, organism groups, or ecological parameters. This has resulted in a science in which there is considerable knowledge of some of the parts but little understanding of the whole.

The philosophy of the studies reported in this volume is different. For over 15 years Professor Szabo and his colleagues have conducted an intensive study of soil microflora and fauna in a single area of oak-hornbeam forest in western Hungary. The concentration on

a single site is deliberate. Only in that way can "those general principles which govern the activities of complex saprophytic communities in the soil" be recognised and understood. It is in that context that the reader is asked to judge the contents of this book.

The first chapter describes features of the forest site at the ecosystem level; in Chapter Two the focus becomes finer and we are given accounts of micro-morphological studies, the utilisation of techniques of soil sectioning and electron microscopy, and the distribution of microbial communities and their microhabitats. The third chapter gives an account of the distribution of soil animals and microorganisms in relation to the conditions of moisture and temperature in the soil.

It is not until Chapter Four, however, that the main subject of this book is encountered: the structure and species composition of the microbial communities occupying selected habitats of the forest-rendzina soil. A discussion of the current status of the species-concept in the Actinomycetes leads on to a consideration of the techniques of multiple character testing and numerical similarity analysis by which Professor Szabo defines the members of his microflora. In this chapter 34 species-groups from the A horizon of the soil are described using those methods; the description is supported by many pages

Spectroscopy . . .

Laser Spectroscopy. By Richard G. Brewer and Aram Mooradian. Pp. xi+671. (Plenum: New York and London, 1974.) \$41.40.

THIS volume contains the proceedings of a Conference held at Vail, Colorado, between June 25 and 29, 1973—one of the most interesting conferences I have had the privilege of attending. The book reports on 45 invited papers and reflects a significant landmark in the application of lasers to fundamental physical investigation in that the impact of tunable laser sources, or related methods, on the spectroscopy of atomic and molecular systems could be seen clearly for the first time. Orders of magnitude advances in resolution and/or spectral brightness were demonstrated and used. The meeting, which was international and interdisciplinary, covered a spectrum from the ultraviolet to the far infrared and microwave regions; it was set in context by addresses covering wider fields of physics by Gerhard Herzberg and Edward Teller.

In some ways visible wavelength, dye lasers are the most highly developed: examples of high resolution (50 kHz) atomic beam spectroscopy, spectroscopy of selectively excited atomic states, saturated spectroscopy eliminating Doppler broadening, and quantum beats—modulations arising from the coherent superposition of excited states observed in fluorescence—are variously reported from Cologne, Columbia, London and Stanford universities.

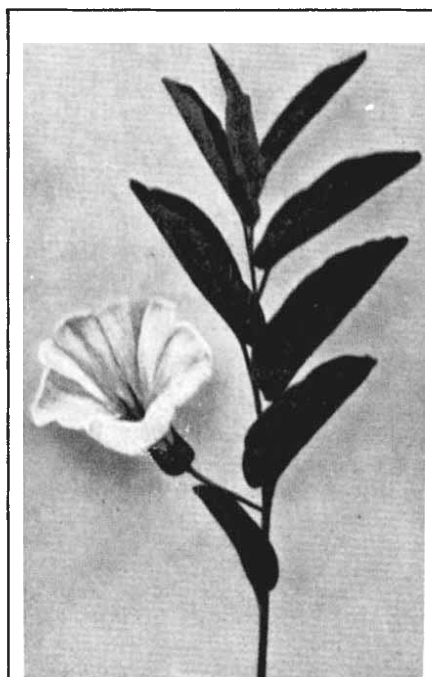
Spectroscopy using tunable infrared lasers is attendant upon the continuing development of the different laser systems. Parametric oscillators (LiNbO_3 , CdSe) now cover the range from 0.6 μm to 11 μm (but not continuously) and, with mixing techniques, reach 18 μm . As reported by Stanford University, however, resolution is so far disappointing—linewidths are only 0.1–1 cm^{-1} —and good spectra are as yet rare. Four-wave parametric mixing in alkali metal vapours, developed by the International Business Machine company, now covers the range 2–25 μm with rather similar properties.

By contrast, semiconductor diode lasers, until now originating mainly from the Lincoln Laboratory, MIT, collectively extend from 1–32 μm and have yielded many high quality spectra though, individually, they cover only a few wavenumbers. These lasers can now be operated with external cavities. Hyperfine splitting, Λ -doubling and Zeeman splitting effects using laser linewidths sometimes as low as 100 kHz are demonstrated. Progress on Spin-flip Raman lasers is reported from MIT, Bell Laboratories and Heriot-Watt

University; this technique retains advantages in its combination of high power and narrow linewidth (from 1 kW at 0.02 cm^{-1} , pulsed, to 1 W at 100 kHz, continuous wave) and is now giving high quality spectra.

Numerous experiments using lasers on molecules and atoms are reported, all reflecting the high precision of measurement now possible. Subjects covered include Lamb-dip spectroscopy, infrared microwave double resonance, precision calibration and stabilisation, heterodyning, laser photochemistry and astrophysical distance measurements. This excellent book is essential for any laboratory concerned with laser spectroscopy.

S. D. Smith



Upright or low bindweed, *Convolvulus spithameus*. From *Wild Flowers*. Revised edition. By Homer D. House. Pp. 362+364 colour plates. (Collier-Macmillan; New York, May 1975.) £5.00.

. . . and spectroscopy

¹³C NMR Spectroscopy: Methods and Applications. By E. Breitmaier and W. Voelter. Pp. xiii+303. (Chemie: Weinheim, 1974.) DM98.00.

THIS book by these authors has obviously grown to adult status from their earlier review in *Angewandte Chemie*. Their child has expanded naturally in each of its parts and has matured considerably but, genes being what they are, is still bound by the same limited outlook, which is strictly organic. Its name, therefore, could be very misleading to the prospective buyer, especially if he detects the strong physical accents of the companion monographs in the family series. Evidently the godfather realised the child's predilections,

since his foreword uses the term 'organic' in every other line, but it is clear that the editor ought to have exerted his influence and judgement at the baptism.

The first three chapters give 20 pages of basic, introductory discussion of nuclear magnetic resonance (NMR), 45 pages on methods, and another 45 on the relationships of structure to NMR data, mainly covering shifts (20 pages) and coupling constants (20 pages) with only 6 pages on relaxation times. The introductory first chapter is rather unbalanced—there is much detail, unused later, concerning the Bloch equations, and arguably not enough on complexities in spectra. It contains annoying, but perhaps trivial, mistakes or views; for example, it is stated that shifts, unlike couplings, are not true structural parameters, and there is a misleading labelling of the spin levels in an AX system. A short discussion of relaxation effects in that system would have been useful. Chapter 2 is good but could have been improved by the inclusion of a section on assignment techniques, if only in summary or even tabular form. In chapter 3 the authors acknowledge that their adopted sign convention for shifts is unusual but they give no reason for so sprinkling their handsome volume with a plethora of expensive negative signs.

The second half of the volume is devoted to applications. There are only two chapters, rather arbitrarily divided into "Organic Compounds" and "Natural Products". The volume contains virtually nothing on carbon-containing ligands in either organometallic or other complexes. Discussions of paramagnetic systems and of the phenomenon of chemically induced polarisation are avoided. The latter, even if it had not been extensively applied to ^{13}C studies at the time of writing the book, was clearly going to prove important even then.

The authors have done reasonably well in avoiding the dangers arising from early abstraction of material and slow book production in a developing field but, inevitably perhaps, occasionally important assumptions are not sufficiently emphasised or even explicitly stated—for example the extreme narrowing condition in the Overhauser section, and the symmetry or motional conditions in the lanthanide shift section.

In spite of these criticisms and the high price, the book has attractions. It is very well produced, pleasingly designed, printed and presented, and has 82 excellent figures.

I should like to congratulate the authors on their clarity of expression, especially as they have written in a language which is not their own.

E. W. Randall

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carposporangium...
flying tuck...
pentaerythritol tetranitrate...
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Young men

Essays on the Nervous System. By R. Bellairs and E. G. Gray. Pp. viii+511. (Clarendon: Oxford; Oxford University Press: London, 1974.) £11.50.

THIS admirable book consists of nearly 20 essays by former pupils and colleagues of J. Z. Young—essays well chosen and substantial. They cover most of Young's fields of interest—endocrine activity in the nervous system, by the late Sir Francis Knowles; learning in the octopus, by M. J. Wells; synaptology, by E. G. Gray; the vertebrate retina, by B. B. Boycott—as well as the hard core of the organisation of the central nervous system represented by Zeki on the visual cortex, Webster on vision in the pigeon, Nadel and O'Keefe on the hippocampus, and Ralston on the spinal cord. The peripheral nervous system includes the muscle spindle described by David Barker—a pupil of Young at Oxford before his University College days—growth of nerve fibres in tissue culture by D. W. James; and Hall-Craggs on mammalian skeletal muscle. P. K. Thomas on nerve injury reminds us of Young's war time interests from which grew new lines of immense importance.

It is an enormous range of interests, in which he has touched little without making it flower. Sometimes the flowers take unexpected shapes. In Zeki's work sharp and detailed localisation appears as the ground plan of the various visual re-representations in the prestriate cortex. In the paper on the hippocampus a new and fascinating model is put forward to account for the detailed and regular structure of that puzzling formation.

This is, in fact, the thread that joins up this variety of contributions—the need for detailed and exact knowledge of structure on which to base any account of function in the nervous system. There was a time when random spread and connection of nerve fibres was believed to occur even in the visual cortex. Since then we have learned of the precise limits of eye dominance and orientation columns in the cortex, the sharply defined decussation of fibre tracts in the octopus, and the orderly growth and regrowth of fibres in the optic tracts and tectum. It is arguable that although there have been great advances in knowledge of synaptology from electron microscopic studies of the retina described here by Boycott and more generally by Colonnier, we still need more information at the optical microscopic level. Golgi stains give us a small sample of the cells present but only as individuals isolated from each other. Perhaps what we need more is something which does not

yet exist—Golgi-like pictures limited to small groups of cells which are immediately linked to one another, instead of being picked out by the vagaries of staining.

This thread runs through Graziadei's account of the curious regeneration to be found in olfactory nerves, in Guillery's account of experimental production of structural changes in visual pathways, and in Webster's valuable account of the present state of knowledge of visual pathways in birds. Rather oddly, it is not obvious in Wall's essay on cognition and perception as a preliminary to relevant behaviour. What better title could one have for Well's account of the octopus than 'A location for learning'?

This book is a worthy tribute to the work and inspiration of one who has moulded twentieth century studies of the nervous system. **D. Whitteridge**

Applied estimation

Applied Optimal Estimation. Edited by A. Gelb. Pp. 374. (MIT Press: Cambridge, Massachusetts and London; 1974.) \$6.95.

THE title of this eclectic compendium is a conventional solecism. 'Applied' means applicable as in 'applied mathematics'. Examples of the use of optimal estimation are included to elucidate the techniques rather than to discuss the application.

A high proportion of computer time in current use is expended on optimally fitting data to models which are ill founded in theory and erroneous in application. Examples abound in every field, from particle physics to town-planning. A mathematically written textbook which sets out conditions under which models can be built up is needed; it may have the same title as this book but this book is not it. The fact that this volume will increase the number of persons technically equipped to estimate model parameters optimally may well turn out to be a misfortune.

But in spite of reservations about what this book is not, it is an excellent handbook of practical, ably described and heuristically presented techniques. It assumes a degree of mathematical sophistication and knowledge which could be reasonably expected in a graduate (the first chapter summarising this information is too breathless to be didactic), treats linear optimisation fairly exhaustively, and makes extensive inroads into non-linear theory. Space is also found for sensitivity analysis and the last chapter on "Additional Topics" includes stochastic estimation and real-time parameter identification.

P. E. Roe

obituary

Arne Westgren, a pioneer in the application of X-ray diffraction to physical metallurgy, and also well-known for his X-ray crystallographic work in other fields of inorganic chemistry, died in Stockholm on March 7, at the age of 85.

Westgren started his academic career in Uppsala as a pupil of The Svedberg and in 1915 obtained the doctoral degree with a thesis on Brownian movement. While employed as a metallurgist at the ballbearing company SKF, he was confronted with several problems which he thought could be solved by X-ray diffraction methods, and found that the α - β transition in iron was not accompanied by any structural change, whereas γ iron showed the same cubic face-centered structure as the austenitic steels at room temperature. These successful results gave Westgren an appointment at the Institute for Metal Research in Stockholm in 1921, where he began an extremely fruitful collaboration with Gösta Phragmén.

(Phragmén's great experimental skill resulted in the construction of very efficient X-ray focusing cameras). The studies of the iron modifications were continued and the structure of δ iron established. The unit-cell dimensions of cementite were also determined. In the copper-aluminium system a phase with the γ -brass structure was found for the first time, although its structure was not then solved. The structural analogies in the systems copper-zinc, silver-zinc and gold-zinc were found by Westgren and Phragmén in 1925. At about the same time they investigated the modifications of manganese and studied the carbide systems of chromium, molybdenum and tungsten. They also succeeded in characterising the 'high-speed' steel carbide. The structural analogies between the three systems copper-zinc, copper-aluminium and copper-tin, found by Westgren and Phragmén, gave more solid support to the rule proposed at the same time by Hume-Rothery, and founded on microscopical observa-

tions. In 1927 he was appointed Professor of General and Inorganic Chemistry at the University of Stockholm. This resulted in an increasing number of pupils, but nevertheless the collaboration with Phragmén lasted until the latter's death in 1944. The work on metallic systems continued, resulting in more complete structure determinations of phases as well as the solving of a considerable number of new structures. Extensive work on non-metallic systems was also carried out in Westgren's laboratory but was to a great extent performed by pupils of Westgren. In 1943 he was appointed Secretary of the Royal Swedish Academy of Sciences, and from that year only had time to turn to X-ray crystallography in leisure moments. He retired from this last appointment in 1959. Westgren also served as Secretary to the Nobel Committees for Physics and Chemistry from 1926 to 1943 and he was Chairman of the Committee for Chemistry from 1944 to 1965.

announcements

Awards

The prizewinners of the **Feldberg Foundation Awards** are **H. G. Wittman** and **J. B. Gurdon, FRS**.

The Chemical Institute of Canada has announced the following awards: **Fisher Scientific Lecture Award** to **S. Barabas** (application of automated chemical analysis in industry); **Montreal Medal** to **B. B. Migicovsky** ("leadership in the profession of chemistry"); **Merck, Sharp & Dohme Lecture Award** to **L. D. Hall** (synthesising novel carbohydrate derivatives and developing heteronuclear magnetic resonance spectroscopy to study organic compounds in solution); **Noranda Lecture Award** to **B. R. James** (contributions to physical and inorganic chemistry by a scientist under 40); **Chemical Education Award** to **W. E. Harris** (modernising university teaching of analytical chemistry).

The National Academy of Sciences (Washington) has announced the following awards: **James Craig Watson Medal** to **G. M. Clemence** (independent

derivation of a new analytical theory of the motion of Mars); **John J. Carty Medal** to **J. T. Wilson** (theory of plate tectonics); **Henryk Arctowski Medal** to **J. M. Beckers** (discovery and study of exotic small-scale phenomena in the Sun); **Steel Foundation Award** to **B. M. Alberts** (isolation of proteins required for DNA replication and genetic recombination, and interaction thereof); **Award for Environmental Quality** to **J. T. Middleton** (adverse effects of photochemical air pollution in agriculturally important plants); **Arthur L. Day Prize** to **D. H. Matthews** and **F. J. Vine** (theory of plate tectonics); **Benjamin Apthorp Gould Prize** to **Lodewijk Woltjer** (Crab Nebula and galactic magnetic fields); **H. P. Robertson Memorial Lectureship** to **M. Rees** (high energy astrophysics and theory of quasistellar sources, radio galaxies and X-ray sources).

H. Marion and **G. Mahfouz** have been awarded **Telford Gold Medals** by the Institution of Civil Engineers for their paper 'Design and construction of the Ekofisk artificial island.'

The Royal Society has announced the election of the following as **Foreign Members**: **H. Gilman** (organometallic compounds and their reactions); **M. Heidelberger** (antigen-antibody interactions and structure of bacterial polysaccharides); **F. Lynen** (intermediary metabolism and enzyme action); **L. Onsager** (statistical physics and chemistry).

L'Académie des sciences, Paris, has elected two foreign associates: **Albert Claude** (Belgium), the winner of the Nobel Prize for Medicine in 1974; and **Joseph Doob** (United States), the mathematician.

Miscellaneous

Research on cancer. The International Union Against Cancer is supporting the Eleanor Roosevelt Fellowships, intended for experienced investigators carrying out independent research at a single institution in another country. Applications and further information (by September 1): International Union Against Cancer, PO Box 400, 1211 Geneva 2, Switzerland.

International meetings

June 23–24, **Scientific and engineering secondary information transfer for developing countries**, Brussels (General Secretary, ICSU AB, 17 rue Mirabeau, 75016 Paris, France).

July 3–10, **International botanical congress**, Leningrad (XII International Botanical Congress, 2 Professor Popov Str, Leningrad 197022, USSR).

July 6–10, **Underwater physiology**, San Diego (Secretariat, 6th Symposium on Underwater Physiology, 9650 Rockville Pike, Bethesda, Maryland 20014).

July 6–11, **Pure and applied chemistry**, Jerusalem (Organising Committee, 25th Congress of the International Union of Pure and Applied Chemistry, POB 16271, Tel Aviv, Israel).

July 6–11, **Nuclear magnetic resonance spectroscopy**, St Andrews, Scotland (Dr J. F. Gibson, The Chemical Society, Burlington House, London W1V 0BN, UK).

July 7–11, **Plant pathology**, London (The Director, Commonwealth Mycological Institute, Ferry Lane, Kew, Richmond, Surrey, TW9 3AF, UK).

July 8–12, **International committee on laboratory animals**, Thessaloniki, Greece (Dr N. Simionescu, Yale University School of Medicine, Cell Biology Department, 333 Cedar Street, New Haven, Connecticut 06510).

July 8–12, **Phonon scattering in solids**, Nottingham, UK (The Institute of Physics, 47 Belgrave Square, London SW1X 8QX, UK).

July 10–11, **Autoradiography**, Edinburgh, UK (Dr J. Jacob, Department of Genetics, West Mains Road, Edinburgh EH9 3JN, UK or Dr T. Appleton, Physiological Laboratory, Cambridge CB2 3EG, UK).

July 10–12, **Inflammation**, Nottingham (Dr Alma B. Simmonds, Chelsea College, University of London, Manresa Road, London SW3, UK).

July 13–18, **Enzymatic and homogeneous catalysis**, Santander, Spain (Dr A. O. Ballesteros, Departamento de Catálisis, CSIC, Serrano, 119 Madrid-6, Spain).

July 13–18, **Clinical chemistry**, Toronto (Dr F. H. Sims, Chairman of the Publicity Committee, Women's College Hospital, Toronto, Ontario, Canada).

July 13–18, **Chemotherapy**, London (Conference Services, 43 Charles Street, London W1, UK).

Reports and publications

Great Britain

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Other Countries

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